Syndrome classification through a retrospective analysis of porcine submissions to a regional animal health laboratory.

by

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Abstract

In response to the global threats of emerging infectious diseases and bioterrorism events, public health surveillance developed analytical methods to cluster early health indicators from multiple data sources into "syndromes" for rapid and efficient disease detection. Syndromic surveillance has become well established in public health, using many different health indicators from multiple sources. In animal health, the timeliness and efficiency of disease detection in early warning surveillance systems has been enhanced by including syndromic surveillance methods. Animal health syndromic surveillance improves disease detection through the analysis of pre-diagnostic data collected for other purposes, from sources such as laboratories, veterinary clinics, abattoirs, farms and pharmacies. However, the data are inherently non-disease specific compared to traditional surveillance and require analyses to ensure that syndromes represent significant diseases as accurately as possible. Syndrome classification is an analytical process that identifies, collates and validates pre-diagnostic indicators within a data source into accurate and viable syndromes.

The goals of this thesis were as follows: a) Review surveillance systems and methods to understand the scale, complexity and validity of different syndromic surveillance approaches. b) Describe and evaluate six years of swine laboratory submission data to Veterinary Diagnostic Services (VDS) in the province of Manitoba, Canada, for the purpose of syndromic surveillance. c) Finally, identify and validate the most appropriate syndromes from pre-diagnostic data within the submitted swine cases.

An initial systematic review of public health syndromic surveillance was conducted with 81 studies meeting the criteria. The variety and frequency of populations under surveillance, information sources, pre-diagnostic indicators, syndromes and reported values were recorded. The predominant methods for syndrome classification, temporal and spatial analysis and aberration detection were also described.

21,665 swine laboratory submissions from January 2003 to March 2009, including 4726 pathology cases, were evaluated. The frequency and distributions of the predominant pre-diagnostic indicators, test requests and specimen types, were described. The most common pathology diagnoses and organ system involvement were reported for the pathology submissions. For syndrome validation, a Multiple Correspondence Analysis was conducted to cluster multiple pathology diagnoses per case into four diagnostic groups based on organ systems; Respiratory, Multisystemic, Gastrointestinal and "Other".

Syndrome classification was completed, first using agglomerative hierarchical clustering to classify syndromes from 30 test requests and 34 specimen types. For validation, the syndromes were used as predictive variables in a multinomial logistic regression model applied to training and test data sets. The overall model sensitivity, specificity and predictive values for each organ system outcome were estimated. The individual syndromes were compared using relative risk ratios and marginal effects. Five syndromes were identified as having a significantly higher predictive association with one organ system group (compared to the other three): Respiratory, GI, Reproductive, Joint and PCV (specific to porcine circovirus associated disease).

The methods in this thesis identified a simplified analytical approach for syndrome classification of laboratory test requests and specimen types within swine submissions. Alternative algorithms for syndrome grouping, establishment of temporal baselines and exploration of automated aberration detection were identified as areas for future research.

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Dedication

This thesis is dedicated to those colleagues whose insight, commitment and professionalism have inspired me throughout my career, and to my family, whose encouragement and understanding made this possible.

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Chapter 1: Thesis Introduction

1.1 Animal Health Surveillance: Descriptions, Purposes and Activities

Effective animal health surveillance is an essential part of evidence based decision making required to protect animal and public health, to provide assurance of a healthy food supply, to support economical and sustainable livestock production and to protect the intrinsic value of animals for the public good (Hasler et al, 2011; Hyder et al, 2011; Lysons et al, 2007). Animal health surveillance provides descriptive information and detailed analysis of animal health hazards in defined populations through systematic (continuous or repeated) measurement (Hoinville et al, 2013). It also links to and informs risk mitigation and promotes intervention with the intent of reducing the overall negative impacts of disease (Hasler et al, 2011) . The core purposes of animal health surveillance can be described as follows; (1) to provide early detection of zoonotic, exotic or emerging disease, (2) to detect change in the epidemiology, pathogenicity and / or infectivity of endemic animal diseases, (3) to substantiate freedom from disease, (4) to describe changes in population risk factors and (5) to define further opportunities to assist in disease control or eradication (Hoinville et al, 2013). The increasing demand for effective animal health surveillance has been driven by the significant negative impacts that animal and zoonotic diseases have had on animal health, public health, the economy and the environment (Lysons et al, 2007). Globally, endemic, emerging, re-emerging and exotic diseases have been occurring with increasing frequency across a greater number geographic regions: bovine spongiform encephalopathy (BSE) foot and mouth disease (FMD), highly pathogenic avian influenza, pandemic influenza, salmonellosis and porcine epidemic diarrhea (PEDv) are recent examples of disease that have had large scale geographic and economic impacts(Gibbens et al, 2008; Hasler et al, 2011; Huang et al, 2013; Hyder et al, 2011; Kosmider et al, 2011). Animal health surveillance involves a wide range of activities, components and systems. Figure 1 represents the most recent concepts and terminology used to describe and evaluate animal health surveillance activities, components and systems (Hoinville et al, 2013; Salman 2003; Stark et al, 2006).

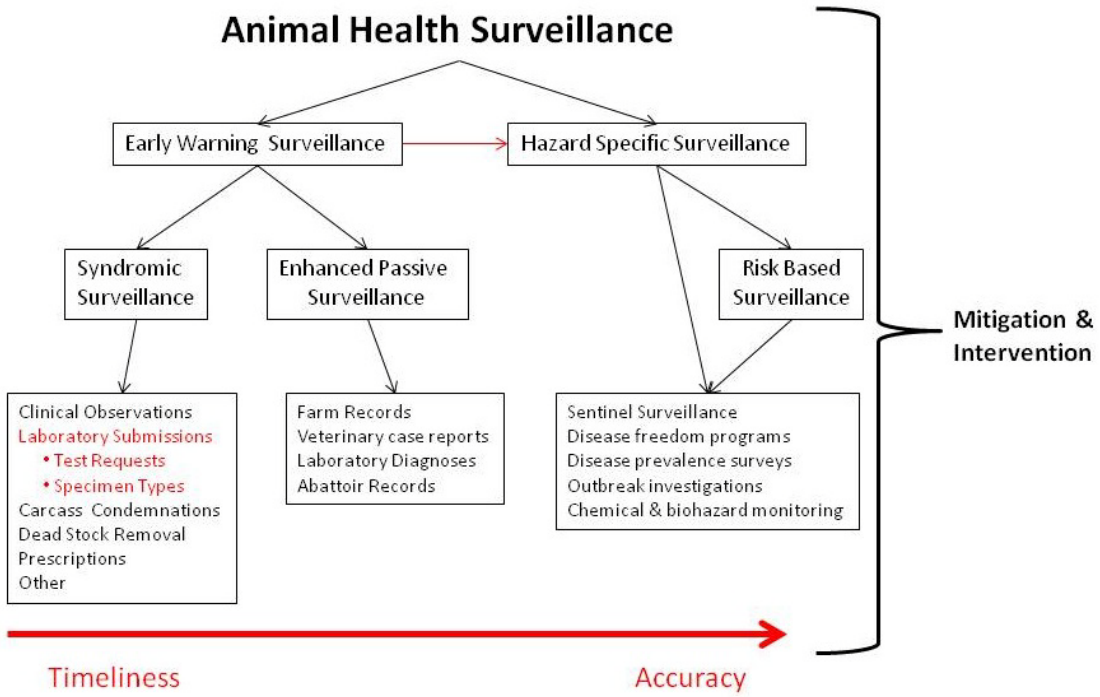

Figure 1: Examples of surveillance activities and components in different surveillance systems.

Hazard specific surveillance encompasses the investigator directed, "active" surveillance methods used in disease control or eradication programs, outbreak response, documentation of freedom from disease for international trade and estimation of the occurrence (prevalence and/or incidence) of endemic diseases within specified regions or time frames. The activities include many established surveillance methods such as systematic serological surveys, sentinel herd testing, hazard or risk factor questionnaires and at risk population testing during outbreaks. Risk based surveillance may be viewed as enhanced methods of hazard specific surveillance where the plan, design and/or the interpretation of results are adjusted by the probability of occurrence and the magnitude of impact (biological and/or economical) of specific health hazards.

Early warning surveillance encompasses surveillance methods that evaluate indicators, reports or observations that occur with routine animal health and economic activities for rapid detection of threats in defined populations or regions. These methods are considered a more timely and efficient means of indentify threats from emerging, re-emerging or exotic diseases, or significant changes of endemic diseases, when compared to hazard specific surveillance (Dupuy et al, 2013a; Hoinville et al, 2013): Early warning surveillance systems and components

are more likely to detect undefined or unexpected threats because the information analysed and the resources used are applied to a broad range of animal health activities and do not focus only on specific hazards. Observer initiated disease reporting through an organized veterinary infrastructure have traditionally been the primary means of early warning surveillance (Kellar, 2005; Lysons et al, 2007; O'Toole, 2010; OIE 2013). The practical application of observer initiated information requires targeted funding, coordination, collation, standardization and analysis by investigators to provide effective and timely surveillance. This integration of observer/investigator roles has led to the proposed definition, enhanced passive surveillance and may be used to inform hazard based surveillance for improved accuracy and for focused intervention and mitigation strategies (Hasler et al, 2011; Hoinville et al, 2013). However, even with integration, traditional early warning surveillance methods have significant limitations in the areas of reporting, time between onset and diagnosis, sensitivity, availability of data and effective use of resources. These issues may be interpreted as the primary reasons why these methods have struggled to keep pace with the increasing global threat of emerging and re-emerging diseases (Dorea et al, 2011; Dupuy et al, 2013a; Kosmider et al, 2011; Shaffer et al, 2008). The expansion of advanced information systems and development of new analytical methods have improved the integration and evaluation of different types of health related indicators from large amounts of data across multiple sources (Bravata et al, 2004; Dorea et al, 2011). New surveillance methods, such as syndromic surveillance have utilized the combination of informatics and analytical tools to improve the timeliness and sensitivity of early warning surveillance systems and components.

1.2 Syndromic surveillance in public and animal health

Syndromic surveillance classifies health related indictors such as clinical observations, laboratory test requests, telephone consultations, internet searches and pharmaceutical sales into pre-diagnostic “syndromes”. Changes in the incidence or prevalence of syndromes are followed over time to rapidly signal potentially detrimental changes in the health of human or animal populations. Effective syndromic surveillance must provide adequate sensitivity and specificity compared to traditional surveillance methods and should improve upon the timeliness of disease detection. The intent is not to replace traditional surveillance, but rather enhance overall surveillance for improvements in investigation and response to significant disease events (Dorea et al, 2011; May et al, 2009). Enhancing overall sensitivity, specificity and

timeliness of disease detection by incorporating syndromic surveillance underscores several key characteristics of these methods: Syndrome classification for the development of sensitive indicators of disease from data that has been collected for other purposes and does not readily indicate any specific disease or group of diseases; temporal and/or spatial analysis that accurately record the occurrence of syndromes from the population under surveillance; aberration detection methods that analyze the temporal and spatial occurrence of the syndromes to detect significant clusters of disease; finally, the validation of the syndromic surveillance methods at determining significant health events from retrospective or prospective data.

Syndromic surveillance methods and systems were developed in public health to detect global threats from emerging infectious diseases, such as severe acute respiratory syndrome (SARS), pandemic influenza and avian influenza, and from bioterrorism threats, such as anthrax (Bravata et al, 2004; Katz et al, 2011). With the ability to employ advanced analytical tools on multiple health related information streams in near real time, syndromic surveillance has become an efficient, rapid and well established means for public health epidemiologists to detect and respond to clusters of disease (Hiller et al, 2013; Hurt-Mullen and Coberly, 2005; Katz et al, 2011). The scope of these systems and methods have expanded beyond emerging disease and bioterrorism threats; greater efficiency has been obtained by including detection of outbreaks in endemic diseases such influenza and acute gastrointestinal diseases (Hiller et al, 2013; Ivanov et al, 2002). Additionally, to increase population coverage and improve "real time" or "near real time" availability of data for analysis, the sources for electronic syndromic surveillance have expanded beyond emergency room and medical clinic sources. Pharmacies, ambulance dispatch, telehealth/telemedicine centres, diagnostic laboratories and medical information web sites are frequent sources (Bravata et al, 2004; Hiller et al, 2013; Hurt-Mullen and Coberly, 2005; Kashiouris et al, 2013). Finally, public health surveillance administrators and researchers have developed guidelines, defined characteristics and established validation methods to determine consistency, applicability and overall performance of syndromic surveillance systems and methods (Kashiouris et al, 2013; Katz et al, 2011; Leal and Laupland, 2008).

In animal health, syndromic surveillance methods have increased with the increasing use of animal health information systems that collect a variety of early disease indicators, including clinical observations, laboratory submissions, carcass condemnations and mortality rates (Figure 1) (Alton et al, 2012; del Rocio et al, 2010; Dorea et al, 2013; Dupuy et al, 2013b; Gibbens et al, 2008; Van Metre et al, 2009; Vourc'h et al, 2006). A recent review of veterinary syndromic surveillance in Europe identified 27 different systems in 12 countries (Dupuy et al, 2013a). These systems include information collected from laboratories, abattoirs, rendering plants, veterinary clinics, farms and pharmacies with the primary objectives of general health surveillance and/or outbreak detection. As in public health, the systems and methods are directed to the early detection of emerging, zoonotic or reportable diseases, or significant changes in endemic diseases (Dupuy et al, 2013a). Also like public health, the initiatives focus on specific ways to conduct syndromic surveillance such as notification of atypical cases, analysis of all clinical cases, or notification of public health concerns such as zoonoses (Dorea et al, 2011; Rabinowitz et al, 2010). Animal health syndromic surveillance has several key challenges that differ from public health: The use, coverage and integration of information systems is not on the same scale in animal health, animal health information systems are often not able to provide data in "real time" (within 24 hours) and globally recognized standardized nomenclature of disease (e.g. World Health Organization's International Classification of Diseases) are not readily available for syndrome development (Bartlett et al, 2010; Dupuy et al, 2013a; Hoinville et al, 2013). However, the opportunities to expand animal health syndromic surveillance and address key challenges are improved through reviews and analyses of the public health systems and methods. Furthermore the "One Health" approach of sharing knowledge and developing synergies between human and animal health provides a framework for implementation of complimentary syndromic surveillance activities that may enhance sensitivity and timeliness of detecting human and animal threats (Dorea et al, 2011; Dupuy et al, 2013a; Shaffer et al, 2008; Vrbova et al, 2010).

1.3 The role of animal health laboratories in traditional and syndromic surveillance.

Animal health laboratories are key components of national veterinary infrastructure that exist in three sectors; public laboratories usually associated with government agriculture departments, schools of veterinary medicine, and private commercial laboratories (Shaffer et al, 2008). Animal health laboratories, veterinary diagnostic pathologists and laboratory

technologists have a primary role of providing diagnostic and pathology services to clinical veterinary practice and food production systems. They also provide an important secondary public veterinary role through diagnostic capacity for hazard specific surveillance and pathology expertise for identification of emerging or re-emerging diseases in traditional early warning surveillance (Gibbens et al, 2008; O'Toole, 2010; Pasick et al, 2007; Schmitt, 2003). To further support both roles, animal health laboratories typically maintain current and historic laboratory data in digital format and provide a limited degree of data standardization; laboratory information management systems (LIMS) contain records in standard formats that include species, specimens submitted, test orders and results, pathology diagnoses, submission date and a geographic references (Shaffer et al, 2008). Additionally, animal health laboratories frequently separate and classify submissions into diagnostic and non-diagnostic reasons, particularly for food animal production (Gibbens et al, 2008). The separation provides further detail where diagnostic reasons support early warning surveillance through disease identification, disease follow up and suspicion of a notifiable disease and non-diagnostic reasons support hazard specific surveillance through health status determination, vaccine response, export or domestic sale, research and notifiable disease screening (Dorea et al, 2011; Schmitt, 2003).

Surveillance activities conducted through laboratories have considerable imperfections such as voluntary under reporting of disease, biases through clinical decisions regarding case submissions and economic factors influencing submission decisions (O'Sullivan et al, 2012; Sintchenko and Gallego, 2009). However, animal health laboratory submissions remain an important component of early warning surveillance as veterinary clinicians and pathologists are more likely to submit and investigate unexpected or unknown adverse animal health events (Gibbens et al, 2008; O'Sullivan et al, 2012; O'Toole, 2010; Zurbrigg and Van den Borre, 2013). The early warning surveillance role has been enhanced through laboratory based syndromic methods involving regional animal health laboratories and over specific animal populations (Dorea et al, 2012; Hyder et al, 2011; Kosmider et al, 2011; Odoi et al, 2009; Shaffer et al, 2008). The role has been further expanded by involving animal health laboratories in broad based veterinary syndromic surveillance initiatives (Dupuy et al, 2013a).

Several key factors have led to the exploration and development of syndromic systems and methods using animal health laboratories:

- Public support through direct infrastructure funding, operational grants or partial coverage of specific case submissions (particularly food animal) provides incentive for access and utilization of core animal health data to support broader public surveillance initiatives.
- Animal health laboratories typically provide centralized service to larger geographic regions and animal populations, providing greater coverage through a single data source when compared to veterinary clinic data. Additionally, national systems for public and animal health have linked animal health laboratories to varying degrees. The linkages have expanded use of surveillance methods, provided multi jurisdictional surveillance coverage and have created greater levels of data standardization (Kloeze et al, 2012; Lysons et al, 2007).
- Pre-diagnostic indicators, such as test requests and specimen types (Figure 1) are available for syndrome development and classification (Dorea et al, 2013; Hyder et al, 2011; Odoi et al, 2009; Shaffer et al, 2008). While not as timely as clinical data, pre-diagnostic laboratory data are often, as noted above, more accessible and is believed to have greater specificity, as it is closer to a final outcome (test results or diagnoses) (Dorea et al, 2011; Shaffer et al, 2008).
- LIMS contain both pre-diagnostic indicators, as well as diagnostic outcomes such as test results and pathology diagnoses (Figure 1) that may be used to estimate syndrome sensitivity. LIMS also provide access to large amounts of data that allow use of advanced analytic tools for classification and cluster detection (Dorea et al, 2012; Hyder et al, 2011; Kosmider et al, 2011; Odoi et al, 2009; Shaffer et al, 2008).
- Within animal health infrastructures, laboratories frequently have data standardization and numerical coding of disease nomenclature that will improve the ability for data classification compared to other information sources. The ability to classify data across laboratories also improves when standard nomenclature is established within an integrated national animal health network (Gibbens et al, 2008; Lysons et al, 2007). However, standardized animal disease nomenclatures remain infrequently used across animal health laboratories even when available through standards such as Standard Nomenclature for Veterinary Diseases and Operations (SNVDO), Logical Observation

Identifiers Names and Codes (LOINC), Health Level Seven International (HL7) or Systemized Nomenclature of Human and Veterinary Medicine (SNOMED) (Bartlett et al, 2010; Dorea et al, 2011).

1.4 The importance of the swine industry and swine health; Global, Canadian and Manitoba perspectives

Pork is a significant food source in many regions of the world, especially in countries where increasing personal wealth and changes in cultural preferences have led to overall increases in the demand for meat and associated livestock production (FAO 2013) . In meeting the demand, global swine production represents the largest volume of world meat production, increasing from 72 to 108 million metric tons between 1993 and 2013 (FAO 2013). The trend is also represented in the global population of swine which has increased from 848 million in 1993 to 977 million in 2013. The majority of swine production occurs in China (49.3%), followed by the European Union (15.0%), North America (8.0%) and South America (6.4%). However, with the exception of China, world swine production has remained constant or with only marginal increases since 2006-2007 in spite of an increasing global population (FAO 2013). North American and Canadian swine production has followed the trends with marginal increases between 1.3 and 1.7% per year over the same time frame (FAO 2013). However increases in sow productivity and increases in carcass size has led to increases in pork produced without concurrent increase in the inventory of pigs held.

The Canadian swine industry, as with swine production in all of North America has significantly changed over the last decade, moving away from smaller mixed independent operations to greater vertical integration and contractual production (Kliebenstein and Lawrence, 1995; MacDonald, 2003). Farms are highly specialized, where there is individual and/or centralized control over the different levels of production, including genetics, feed supply and even slaughter capacity. The Canadian hog industry is the fourth largest agriculture industry in Canada, worth 9.8 billion annually and represented by 12.7 million head on 7,341 farms (Brisson Y 2014). Canada is the fifth largest global exporter of pork, at just over 1 million tonnes. The United States is the primary importer of pork and live pigs from Canada at over 25% of total swine industry exports (Brisson Y 2014). Other significant importers of Canadian pork are China, Japan, Russia and South Korea. The province of Manitoba contains a significant

proportion of the Canadian swine industry: Manitoba swine producers export the most live pigs and have developed the third largest pork industry overall in Canada. The Manitoba swine industry has 590 farms on approximately 1100 sites and is the largest livestock industry in the province. Total annual production is approximately 8 million pigs per annum with 2.6 million held on farms at any given time and a sow herd of 318,000. It represents a total value to the Manitoba economy of $1 billion or 1.5% of Gross Domestic Product (Brisson Y 2014; Honey J 2012).

From 2006 to present, the Canadian and Manitoba swine industries have experienced significant challenges due to economic, trade and environmental impacts, as well as disease occurrences. Manitoba swine production has been particularly affected due to the high degree of export dependency and a province wide government moratorium on industry development (Anon 2007; Honey J 2012; Whiting et al, 2011). Major contributing factors preventing growth include an elevated Canadian/US exchange rate, trade disruptions such as Country Of Origin Labeling (COOL), elevated feed prices related to drought conditions and high oil prices, and the public perception of the environmental impacts of large scale livestock production (Brisson Y 2014; Honey J 2012; Thevenaz 2011).

As with other animal populations, swine populations in Manitoba, Canada and globally have been increasingly affected by emerging, endemic and zoonotic diseases(Amezcua et al, 2013; Dupuy et al, 2013a; Vourc'h et al, 2006). Over the past 15 years, Manitoba and Canadian swine health and production have been impacted by diseases such as porcine circovirus associated disease (PCVAD), porcine reproductive and respiratory syndrome (PRRS), swine influenza (SIV) pandemic influenza (panH1N1) and porcine epidemic diarrhea (PED) (Carman et al, 2008; Gagnon et al, 2007; Huang et al, 2013; Pasma, 2008; Pasma and Joseph, 2010; Poljak et al, 2010; Young et al, 2010). The disease impacts, along with a strong focus on high health standards and the greater degree of vertical integration has led to a considerable use of veterinary infrastructure and increasing reliance on animal health surveillance (Amezcua et al, 2013; O'Sullivan et al, 2012; Pasma and Joseph, 2010;Verdon et al. 2012). In Canada, specialized private clinical practice, with public veterinary coordination and diagnostic laboratory services form the core components of surveillance (both early warning and hazard specific), risk mitigation and intervention. This is well represented in Manitoba, where

veterinary services to the swine industry are through a combination of industry employed veterinarians, independent swine only practice and mixed animal practitioners. Swine health services are further supported by public sector components such as the provincial Chief Veterinary Office (CVO) and the federal Canadian Food Inspection Agency (CFIA). Laboratory services are primarily provided through a full service regional animal health laboratory, Veterinary Diagnostic Services (VDS) with additional services provided by university and private enterprise. VDS and the CVO are part of the Agri-Industry Development and Innovation branch of the provincial department of Agriculture, Food and Rural Development (MAFRD). VDS, with veterinary clinics, and the CVO form the veterinary infrastructure in Manitoba responsible for surveillance, risk mitigation and intervention of endemic, provincially notifiable and emerging diseases within the province. The provincial veterinary infrastructure is also an integral part of the Canadian Animal Health Surveillance Network, a network of federal, provincial and university laboratories, public veterinary agencies and animal health research groups that focuses on the early detection of zoonotic and notifiable diseases (Kloeze et al, 2010).

The purpose of the thesis is to explore the opportunities to expand the surveillance capacity for swine health in the province of Manitoba using syndromic methods. The focus will be on data collected through a regional animal health laboratory, VDS. VDS meets the key factors described above for exploration of syndromic surveillance: VDS is a part of the public infrastructure and public funding provides 70% of the financial costs for all diagnostic and non-diagnostic testing conducted at the laboratory on livestock and poultry from farms within Manitoba. Public funding ensures access to core information collected by VDS. Public funding also provides incentive for regular use by veterinary practitioners for diagnostic services provided to Manitoba swine herds. While out of province laboratory services may be utilized for specialized testing, the centralization, proximity and public funding for diagnostics makes VDS the primary diagnostic service provider to the Manitoba swine industry. This is especially true for the rapid necropsy and histopathological services necessary for investigating disease events. VDS has well established case submission protocols that ensure laboratory submissions occur under the supervision of clinical veterinarians and are reflective of singular health events occurring within defined groups of animals. The core case submission data along with the diagnostic and pathological outcomes are maintained within the VDS LIMS. Veterinary selected pre-diagnostic indicators (test requests and specimen types) indicative of significant animal

health events can be effectively accessed for syndrome development and subsequent comparison to final outcomes. Finally, while VDS does not follow any particular standardized disease nomenclature, standard methods for diagnostic reporting and for pathology diagnoses have been incorporated in the LIMS.

The thesis has involved an extended progression from 2009 to present that included a review of syndromic methods, data description and collation, syndrome development, and analysis of syndrome classification. The second chapter represents a systematic review and description of the initial public health syndromic surveillance methods that are the basis for many syndromic methods and systems used in public and animal health. Chapter 3 provides a description of pathology and non-pathology submissions to VDS from 21665 swine submissions over a six year period from 2003 to 2009. This chapter also includes Multiple Correspondence Analysis (MCA) and hierarchical clustering exercises to collate final pathology diagnostic outcomes into four key groups. The fourth chapter is a description of unsupervised learning method, agglomerative hierarchical clustering, to classify pre-diagnostic indicators from pathology cases into syndromes. The chapter also provides a multinomial logistic regression analysis of syndrome predictions compared to grouped pathology outcomes from Chapter 3, using both a sample and a test dataset. The chapter concludes with syndrome validation conducted using relative risks and predictive probabilities. The thesis conclusion (Chapter 5) provides a discussion of the methods applied in the preceding chapters, including their utility and limitations in the context of syndrome classification for animal health surveillance. Future potential steps for animal health syndromic surveillance from laboratory data in Manitoba are included, specifically opportunities for temporal analyses and aberration detection utilising the syndrome classifications.

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Chapter 2: Systematic review of syndromic surveillance in public health

2.1 Introduction

Effective organized and ongoing animal health surveillance is thought to improve the detection of and the response to zoonotic, reportable or emerging disease or changes in the epidemiology, pathogenicity and / or infectivity of endemic animal diseases. Effective animal health surveillance covers a wide range of activities including both passive and active methods such as surveys, sentinel practices, clinical observations, laboratory diagnostics and abattoir monitoring.

Pre-diagnostic (or syndromic) surveillance has been developed and utilized as a method of early disease detection in public health for greater than a decade. Essentially, this is the grouping of data from non-traditional sources, often collected for other purposes, into meaningful classifications (or syndromes) that can be monitored for temporal and spatial change. The primary purpose is for rapid detection of disease often before any specific diagnosis can be made (Leal and Laupland, 2008; van den Wijngaard et al, 2008).

The key components of any syndromic surveillance method are the classifications (or make up) of the syndromes, the analysis of temporal and spatial change in these syndromes, the methods of detecting significant aberrations in temporal or spatial changes and validation of the surveillance methods in determining significant health events.

In veterinary medicine, disease surveillance has focused on regulatory programs and efforts to eradicate specific diseases (Kellar, 2005; Shaffer et al, 2008). To achieve greater efficiency and prioritization, animal disease surveillance has made considerable improvements by focusing on "at risk" populations or activities (Paiba et al, 2007; Stark et al, 2006). Access to "at risk" populations or activities relies on the availability and selection of potential information sources that represent these populations or activities. Animal health data collected for purposes other than surveillance, from a variety of sources, may be useful to detect significant health events from these populations or activities. Furthermore, the increased use of information management systems for data collection, storage and access has increased the amount, type and availability of information available for animal disease surveillance (Paiba et al, 2007). Identification and classification of significant indicators within these data sources is necessary

for effective use, making pre-diagnostic methods an appealing approach. Syndromic surveillance has not been used extensively in the veterinary context but has been recognized as a valid approach to the detection of emerging and zoonotic diseases, as a method for determining change in endemic disease and as an important contributor to public health surveillance. (Gibbens et al, 2008; Glickman et al, 2006; Vourc'h et al, 2006)

One of the information sources that may advance animal health surveillance is submission information to veterinary diagnostic laboratories. It is hypothesized that animal health laboratories not only contain data essential for animal health diagnostics but also for rapid detection and a potentially early response (Gibbens et al, 2008; Lysons et al, 2007; Schmitt, 2003). While it is recognized that laboratory submissions are only part of the veterinary contact with adverse animal health events, it is assumed that in a risk-based manner, veterinary practitioners are more likely to submit to a diagnostic laboratory when unexpected or unknown adverse health events are presented to them.

In public health, a considerable amount of effort has been directed towards syndromic surveillance to rapidly detect emerging diseases or bioterrorism attacks. Recent disease threats from severe acute respiratory syndrome (SARS) and influenza and from bioterrorism events such as the 2001 anthrax attacks in the United States have demonstrated the need for rapid detection and response (Bravata et al, 2004). Recent research into public health surveillance has focused on pre-diagnostic or syndromic surveillance. Key areas under study include accuracy in detecting significant events, classification of non-traditional data into useful syndromes, methods for monitoring syndromes over space and time and methods for determining significant events or signals. The purpose of the following study was to gain understanding of the important aspects of syndromic surveillance in public health for the development and application of syndromic methods to animal health data. The review included areas such as: populations and associated data sources under study, methods of syndrome classification, methods of aberration detection, temporal and spatial analysis, and overall usefulness as a method of rapid disease detection. The review was conducted as a significant part of the background steps taken by the author between January 2009 and March 2010 to conduct an evaluation and analysis of an animal health laboratory database for the purposes of syndromic surveillance.

2.2 Methods

2.2.1 Study description

Published, peer reviewed studies involving syndromic surveillance in public health were sought to contrast and compare different approaches in data sources, populations, primary diseases / syndromes of interest, methods of classification and detection, statistical modeling and reported values. The United States Centre for Disease Control developed guidelines which include a checklist for evaluating public health surveillance systems (Buehler et al). The guidelines cover many broad categories that exceeded the specific objectives of this review. The guidelines did provide input on how to evaluate surveillance system for timeliness, to look for positive predictive value (PPV), sensitivity and specificity. However, the guidelines do not focus on methods of classification and analysis, which were also of interest in this study. This review followed a systematic process; two databases of peer reviewed articles (Medline and CAB abstracts) were searched in July 2009 and March 2010 using an identical search strategy. For this study, the key topics of interest were: Description of the population under study and the associated data sources, the methods of syndromic surveillance conducted, the syndromes or pre-diagnostic indicators under surveillance, the analyses conducted and the reported outcomes. A Boolean method was used to search the databases where the four broad categories listed above were linked by "AND" and the individual search terms within each category were linked by "OR". The search terms are presented in Table 1. Many of the search terms have similar meanings and are more simply described by general terms (see bolded terms in Table 1). However, to limit the possibility of missed articles during database searching, each search term within each category was linked individually.

2.2.2 Study selection and data abstraction

Titles and abstracts were reviewed under the following relevance screening criteria: Study purpose, descriptions of population under surveillance, descriptions of the types and sources of data used, syndromic or pre-diagnostic indicators, recording of spatial and/or temporal information, methods of analysis and reported results. Only primary research articles that focused on public health surveillance through syndromic or pre-diagnostic methods were included. Traditional surveillance methods, including surveys, were included if a pre-diagnostic method (e.g. clinical diagnoses) was part of the method and analysis. Studies with the following

were excluded: Clinical trials and case prediction scoring systems for specific diseases, surveillance system descriptions with no further discussion on surveillance methods or analyses, and algorithm descriptions (classification or aberration detection) without examples from real or simulated data.

Table 1: Search terms for syndromic surveillance in public health.

Population and data sources	**Hospital** (general, public, private, emergency, medical clinic, doctor's office) **Emergency services** (Emergency ward, trauma center, ambulance, emergency phone system), **Pharmacy** (clinical, hospital) **Telemedicine, Telenursing, Medical information system, Laboratory** (clinical, hospital, core) **Health care utilization, Health center, Intensive care, Nursing home, Work place, School** (university, college, public school, day care)
Methods of syndromic surveillance	**Surveillance** (disease, syndromic, diagnostic, pre-diagnostic, electronic), **Health survey, Medical informatics** (public health, medical data processing), **Geographic information systems, Outbreak/epidemic detection, Algorithm** (classification, learning) **Information processing** (artificial intelligence, bioinformatics, information system, causal modeling, computer analysis, computer prediction, computer simulation, constant comparative methods, critical incidents methods, content analysis, data analysis, system analysis, data extraction, data synthesis, decision tree, machine learning)
Pre-diagnostics /Syndromes under surveillance	**Symptomology** (syndrome, symptom, clinical feature, disease marker), **Case definition, Non prescription pharmaceuticals** (utilization, sales of), **Hospital admission, Absenteeism, Laboratory** (submission, diagnosis, result)**, International Classification of Diseases, Diagnostic tests, Prescription, Bioterrorism, Emerging disease, Infectious disease** (communicable, tropical, viral, bacterial, zoonotic), **Zoonosis**
Analytical methods and outcomes reported	**Evaluation, Statistical analysis,** (spatial, temporal, regression, model, etc), **Geographic distribution, Validation, Specific measures** (sensitivity, specificity, positive predictive value, ROC, Area under ROC, WSARE, BCD, BARD, Statistical process control)

To generate descriptive statistics, a database was established using Access 2007 (Microsoft Corp WA), to collect data from articles that passed the relevance screening criteria for the purpose. The data was grouped into seven broad categories with specific topics addressed in each category (see Table 2). The categories chosen were similar to those evaluated by other researchers (Bravata et al, 2004) or as key categories for evaluating syndromic surveillance (Buehler et al). Focus was placed on collecting descriptive data for categories deemed relevant to advance animal health syndromic surveillance, specifically types of data sources used and information collected in broad categories of analysis. Multiple topics in each category were recorded if present in each study.

Data sets that were restricted to a specific demographic were identified as not directly representative of the general population. For example, data sets from public schools, military hospitals or pediatric hospitals, while possible to consider as proxies for the general population, were identified as separate from the general population. No occupation status was assigned to cases from neonatal or pediatric hospitals databases.

Table 2: Categories for collection of data from relevance screening	
Categories	**Topics**
I. Study Demographics	
1. Location	City or Region, State or Province, Country
2. Population	General or Gender specific, Elderly, Adult, Youth, Child All Occupations or Health care, Military/police, Education/social services, General trades, Other
II. Information sources	
1. Data Purpose	Infectious disease, Emerging disease, Bioterrorism event, Accident/injury, Metabolic disease, Other
2. Data Class	Emergency room, Clinic/outpatient, Laboratory, Pharmacy, Telephone help, Internet help, Other
3. Data Source	Single data source, multiple sources
III. Syndrome Classification	Chief complaints, number of complaints, complaint description, Types of coding (ICD-9-CM, Laboratory, clinical findings, other),
IV. Syndromes	Number of syndromes, descriptions
V. Analyses Components	Classification, Temporal, Spatial, Aberration detection, Validation
VI. Analytical Methods	
1. Classification	Supervised or unsupervised methods, Bayes classifier, professional opinion, regression analysis, MCMC
2. Temporal	Time series, ARIMA, EMWA, space-time scan statistic
3. Spatial	Spatial clustering, space time scan statistic
4. Aberration	WSARE, BARD, EARS, CUSUM, Other
VII. Reported values	Frequency, Probability, OR, RR, Kappa, Sensitivity, Specificity, PPV, ROC curve, Area under ROC Curve, Correlation Coefficient, Time intervals, Cusum values

Descriptive statistics were generated for the seven categories and where applicable, compared across categories. Core pieces of data were tabulated and compared using statistical software (Stata 11, StataCorp 2009) for key descriptive pieces.

2.3 Results

A total of three hundred thirteen (313) articles were identified through the search protocol. Due to the relatively small number of articles received through the initial search, each article

was screened once in a systematic fashion. Review of the abstract and title effectively removed the clinical trials, review articles and most system or algorithm descriptions that did not use data to evaluate. Review of the materials and methods and results removed articles without a pre-diagnostic component or those not directed towards public health surveillance. Subsequent to this assessment, a total of eighty-one articles met the inclusion criteria and were subjected to detailed evaluation. The primary areas of focus for surveillance in the articles that met criteria were multiple sources within large urban centres; thirty-nine articles reported surveillance systems or methods with broad coverage in twenty-one urban centres; ten centres within the United States and eleven centres in other countries including Australia (two), Canada (two), China, France, India, Italy, Japan, South Africa and the United Kingdom. The urban centres of Boston, MA and New York, NY were the most reported with seven studies each, evaluating a variety of surveillance systems and methods. Ten articles reviewed surveillance methods from single sites, primarily hospital emergency rooms or health care clinics with five of the ten relating to US hospitals. Public health surveillance methods and systems specifically covering US states (seven), US counties (seven) and an Australian state (one) were also evaluated. Finally, several large multi-region or national surveillance methods were evaluated including six within the US and one each in Australia, French Guiana, Israel, Italy, Japan, Singapore and Taiwan.

2.3.1 Study Demographics

The population descriptions were as follows: seventy-two (89%) studies had adequate descriptions of the populations represented by the data (Aggarwal and Kumar, 2004; Ang et al, 2005; Ansaldi et al, 2008; Besculides et al, 2005; Betancourt et al, 2007; Boak et al, 2008; Bourgeois et al, 2006; Burr et al, 2006; Carrico and Goss, 2005; Chapman et al, 2004; Chapman et al, 2005a; Chapman et al, 2005b; Chen et al, 2005; Chen et al, 2006a; Clothier et al, 2005; Clothier et al, 2006; Cooper et al, 2009; Das et al, 2003; Das et al, 2005; Davies and Finch, 2003; Dembek et al, 2004; Derby et al, 20050; Flamand et al, 2008; Fleischauer et al, 2004; Ford et al, 2007; Greenko et al, 2003; Guasticchi et al, 2009; Haodo et al, 2005; Heffernan et al, 2004; Hoabo et al, 2005; Hogan et al, 2007; Irvin et al, 2003; Ivanov et al, 2002; Ivanov et al, 2003; Jefferson et al, 2008; Kaufman et al, 2007; Kawana et al, 2006; Kleinman et al, 2005; Kulldorff et al, 2007; Lazarus et al, 2002; Lemay et al, 2008; Lenaway and Ambler, 1995; Levin and Raman, 2005; Lewis et al, 2002; Magruder et al, 2005; Mathews et al, 1998; Mikosz et al, 2004;

Moore et al, 2008; Murphy and Burkom, 2008; Muscatello et al, 2005; Nordin et al, 2004; Ohkusa et al, 2005; Osaka et al, 2002; Overhage et al, 2008; Pattie et al, 2009; Piriyawat et al, 2002; Pokorny et al, 2006; Reis and Mandl, 2003; Reis and Mandl, 2004; Scholer et al, 2007; Sloane et al, 2006; Steiner-Sichel et al, 2004; Tappero et al, 1996; Terry et al, 2004; Tokars et al, 2009; Travers et al, 2007; Turner et al, 2006; van den Wijngaard et al, 2008; Wagner et al, 2004; Wang et al, 2005; Wang et al, 2006; Wu et al, 2008; Yin et al, 2008). Of these, fifty-eight studies utilized data sets that were representative of general populations in the areas where they occurred. Fifty-eight studies utilized data sets that were representative of all age groups in the areas where they occurred. The remaining fourteen studies were limited to specific age groups: Six were limited to adults either because they utilised individual prescriptions, were specific to active military personnel or focused on sexually transmitted disease syndromes (Aggarwal and Kumar, 2004; Chen et al, 2006a; Davies and Finch, 2003; Jefferson et al, 2008; Mathews et al, 1998; Yin et al, 2008). Eight studies were specific to children and / or youth; six involved data from neonatal or pediatric hospitals (Bourgeois et al, 2006; Ford et al, 2007; Ivanov et al, 2003; Levin and Raman, 2005; Reis and Mandl, 2003; Wang et al, 2005) and two used data from public school systems (Besculides et al, 2005; Lenaway and Ambler, 1995). Seven studies were limited to specific occupations; four were military personnel or their immediate families (Chen et al, 2006b; Jefferson et al, 2008; Lewis et al, 2002; Pattie et al, 2009), two represented students in public education (Besculides et al, 2005; Lenaway and Ambler, 1995), and one represented a single trade (market vendors) (Yin et al, 2008). One study was directed to women in rural populations (Aggarwal and Kumar, 2004), representing all occupations, but only adults. Two studies represented all age groups but were specific to military personnel and their families (Lewis et al, 2002; Pattie et al, 2009). The majority of studies had data that represented all populations. Nine studies used primarily simulated data sets or modeling and therefore did not represent distinct populations (Barthell et al, 2004; Burkom et al, 2005; Fricker, Jr. et al, 2008; Gierl and Schmidt, 2005; Hutwagner et al, 2005a; Hutwagner et al, 2005b; Kleinman and Abrams, 2006; Kleinman and Abrams, 2008; Wong et al, 2003).

2.3.2 Information sources

The primary classes of data source identified in the selected articles are listed in Table 3. Twenty-five articles identified more than one data class included for surveillance. Emergency

department and critical care data were the most common class reported. Miscellaneous data classes included data from house calls (Flamand et al, 2008), mortality records (Boak et al, 2008), school absenteeism (Besculides et al, 2005; Lenaway and Ambler, 1995) work absenteeism (van den Wijngaard et al, 2008), medical insurance use (Gierl and Schmidt, 2005; Lazarus et al, 2001; Lazarus et al, 2002; Nordin et al, 2004; Tokars et al, 2009), medical locum service (Clothier et al, 2006; Turner et al, 2006), hospital discharge (Ford et al, 2007), disease specific monitoring (Aggarwal and Kumar, 2004; Kawana et al, 2006; Overhage et al, 2008; Pattie et al, 2009; Piriyawat et al, 2002), and ambulance dispatch (Greenko et al, 2003). Thirty-nine articles reported more than one data source, with either single or multiple data classes. Eighteen studies utilized multiple data classes and multiple data sources (Boak et al, 2008; Burkom et al, 2005; Clothier et al, 2005; Clothier et al, 2006; Davies and Finch, 2003; Greenko et al, 2003; Ivanov et al, 2003; Lemay et al, 2008; Levin and Raman, 2005; Lewis et al, 2002; Magruder et al, 2005; Murphy and Burkom, 2008; Overhage et al, 2008; Pattie et al, 2009; Piriyawat et al, 2002; Tokars et al, 2009; Turner et al, 2006; van den Wijngaard et al, 2008). Studies with multiple data sources and classes commonly conducted comparisons for effective surveillance between the data classes or used one data class as a "gold standard" to validate surveillance with another data class (e.g. Laboratory confirmation of clinical diagnoses). Twenty-one studies used a single data class from multiple sources (Barthell et al, 2004; Besculides et al, 2005; Betancourt et al, 2007; Carrico and Goss, 2005; Chapman et al, 2005a; Das et al, 2003; Das et al, 2005; Dembek et al, 2004; Ford et al, 2007; Heffernan et al, 2004; Kaufman et al, 2007; Mikosz et al, 2004; Moore et al, 2008; Muscatello et al, 2005; Ohkusa et al, 2005; Osaka et al, 2002; Reis and Mandl, 2004; Steiner-Sichel et al, 2004; Terry et al, 2004; Wagner et al, 2004; Wu et al, 2008). Databases and electronic records from multiple hospital emergency departments in an urban centre or region was the most common occurrence of a single data class with multiple sources. Seven studies used a single data source with multiple data classes (Bourgeois et al, 2006; Derby et al, 20050; Guasticchi et al, 2009; Kawana et al, 2006; Kulldorff et al, 2007; Mathews et al, 1998; Yin et al, 2008). Clinic, laboratory and miscellaneous data classes were commonly included in single regional data sources managed by a single agency or health authority.

Table 3: Data classes in syndromic surveillance						
	All studies (N=81)		Studies with multiple data classes (N=25, % of all studies		Studies with multiple data sources (N=39), % of all studies	
Data Class	Freq	Percent	Freq	Percent	Freq	Percent
Emergency / Critical care	50	61.7%	16	32.0%	29	58.0%
Clinic / Outpatient	21	25.9%	17	81.0%	14	66.7%
Laboratory	13	16.1%	12	92.3%	8	61.5%
Pharmacy	7	8.6%	4	57.1%	6	85.7%
Telemedicine	6	7.4%	4	66.7%	1	16.7%
Internet Medical	1	1.2%	1	100.0%	1	100.0%
Miscellaneous	20	24.7%	11	55.0%	12	60.0%

A comparison of the most common data classes combined for surveillance purposes is presented Figure 1. It was evident that emergency room data was not commonly combined with other data classes. Emergency room data (with or without laboratory or miscellaneous data classes) was combined with clinic / out patient data types in ten studies, with laboratory data in seven studies and miscellaneous data types in nine studies. Clinic and laboratory studies were often combined as noted in nine of the thirteen studies that included laboratory data. As noted above, this was often for comparison purposes. Miscellaneous data, when combined, were combined with emergency room or clinic data with exception of one study when state mortality records were compared to internet death notices. All four common data classes were combined in four studies.

Interestingly many data types which were considered alternative to primary health care data, such as telemedicine, pharmacy data and most of the miscellaneous data types were not necessarily combined with primary health care data for surveillance purposes. When primary health care data types were included with alternate data types, it was often to estimate the predictive values and sensitivity of the alternate data types.

In the United States, the Electronic Surveillance System for the Early Notification of Community-Based Epidemics (ESSENCE II) is a broad-based public health surveillance system that utilized multiple data types and sources (Lombardo et al, 2003). Many of the studies have analyzed or utilized components or syndrome groupings from ESSENCE.

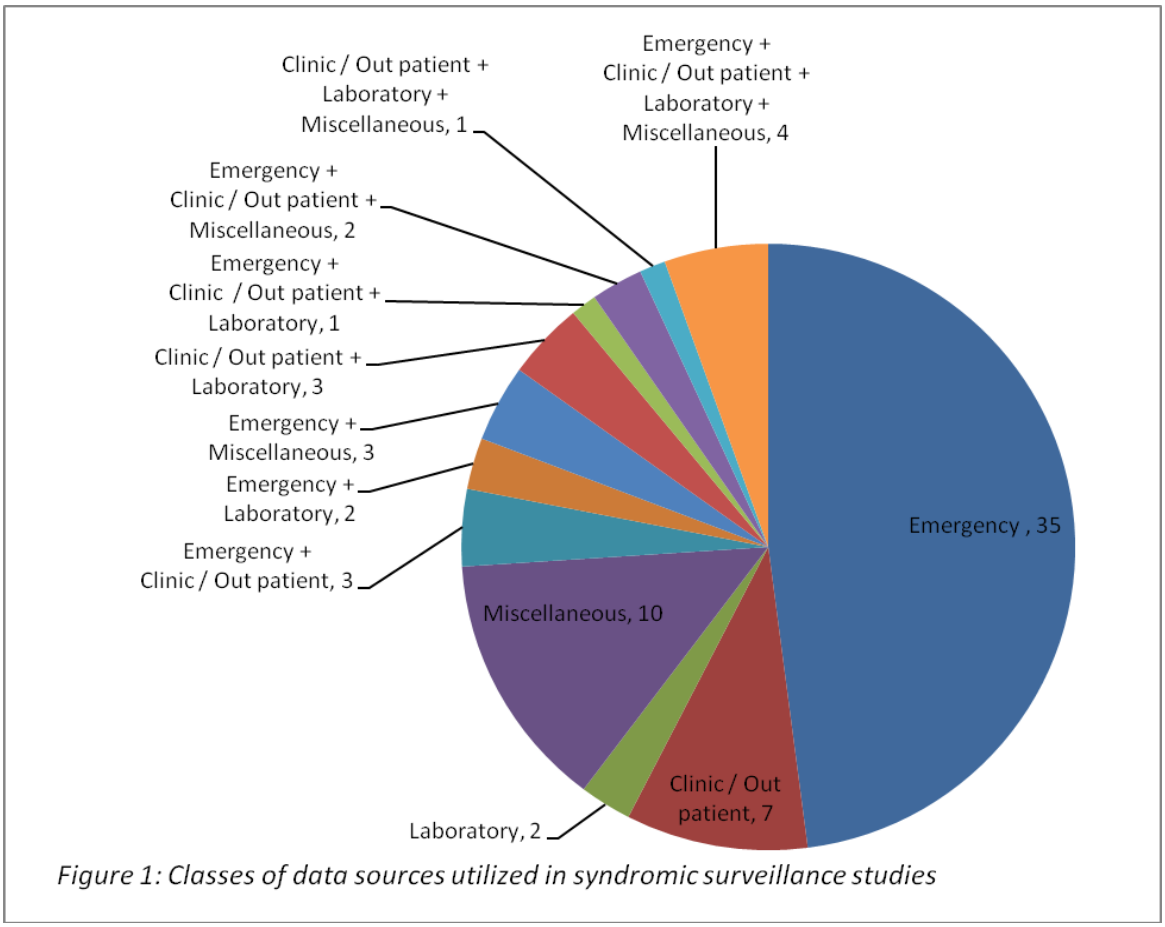

Figure 1: Classes of data sources utilized in syndromic surveillance studies

Three studies that used simulated data did not describe the simulations representing any particular type or source, such as emergency room data (Fricker, Jr. et al, 2008; Kleinman and Abrams, 2006; Kleinman and Abrams, 2008).

The primary disease categories under surveillance in all studies were infectious diseases, bioterrorism events, emerging diseases, metabolic disease and accidents / injuries. Infectious diseases were by far the most common disease category under surveillance across all disease categories in all studies and focused on diseases known to be present in the area and population undersurveillance (Figure 2). The category represented either a broad base of infectious diseases such as respiratory or gastro intestinal viruses or was specific and directed towards diseases such as influenza, salmonellosis, measles, or others. The most common bioterrorism agent under surveillance was anthrax, in particular the respiratory form. Surveillance for emerging diseases focused primarily on SARS or pandemic influenza. The other

disease category encompassed many other health conditions, such as mortality (cause not identified), drug/alcohol abuse and poisoning.

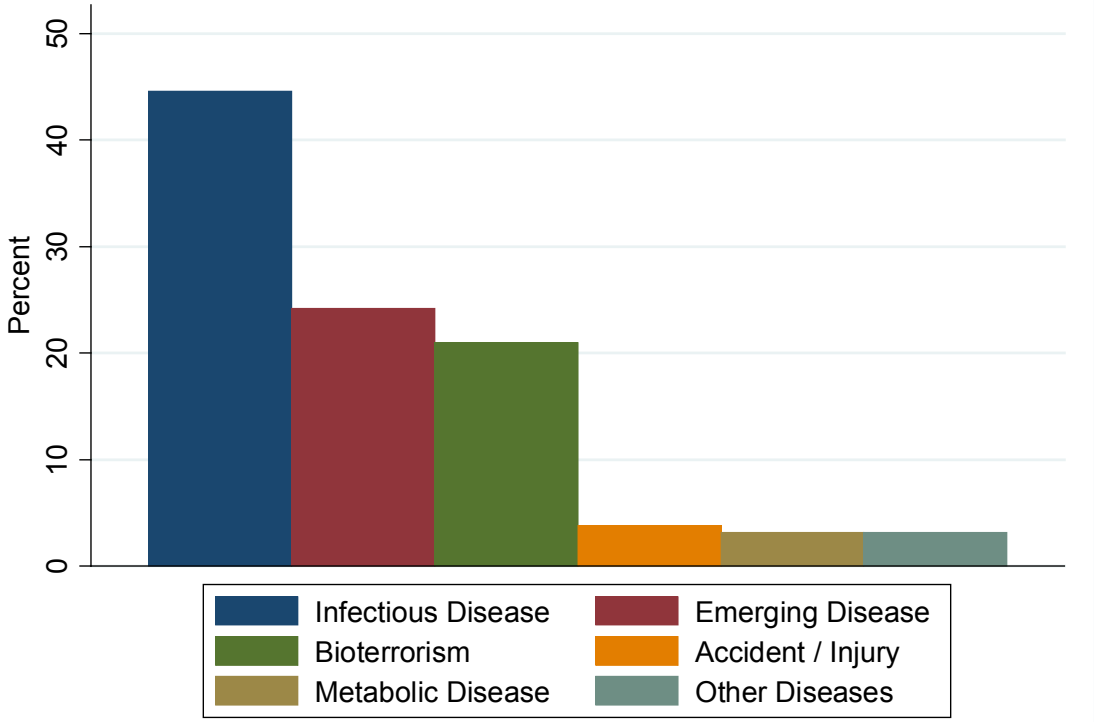

Figure 2: Percent of disease classes under prediagnostic surveillance

2.3.3 Syndrome classification and Syndromes

Syndromic / pre-diagnostic surveillance methods in public health surveillance often utilized chief complaints to assign cases to specific syndromes, especially those that involved primary or front line medical care, such as emergency departments, outpatient clinic or telemedicine. Systems that utilized chief complaints either collected all complaints to assign to specific syndromes (15 studies) or limited what chief complaints were captured (40 studies) (Table 4). When no limits were placed on chief complaints, the number of chief complaints routinely collected or the most common chief complaints were not often reported. Surveillance that limited the number of chief complaints recorded focused primarily on respiratory complaints linked to either infectious diseases (e.g. influenza) or bioterrorism events (21 studies). Other chief complaints routinely captured were linked to gastrointestinal, a combination of gastrointestinal and respiratory, fever and sexually transmitted diseases.

Inclusion of limits on chief complaints was consistent with a limited number of syndromes available for surveillance (primarily a single syndrome), while use of all chief complaints typically led to greater number of syndromes under surveillance (Table 4, Figure 3).
The overall number of syndromes under surveillance for each study was recorded. Seventy-three studies reported the number of syndromes with a mean of 4, a maximum of 15 and a minimum of 1 (Table 4). Forty-four studies included definitions of the syndromes under surveillance. Twenty-nine studies referenced descriptions of the syndromes but did not specifically include them. Eight studies analysed pre-diagnostic surveillance methods but did not describe or report the number of syndromes under surveillance. These studies focused on different methods of detecting significant changes in temporal or spatial results; four studies evaluated aberration detection methods and used simulated data (Fricker, Jr. et al, 2008; Hutwagner et al, 2005a; Kleinman and Abrams, 2006; Kleinman and Abrams, 2008) that generated signals for detection. The four remaining studies compared different detection and reporting methods from existing data that had generated prior signals (Burkom et al, 2005; Gierl and Schmidt, 2005; Overhage et al, 2008; Wong et al, 2003). Thirty-eight studies reported one syndrome for surveillance purposes, as noted above, single syndromes were primarily respiratory, directed most commonly at influenza, SARS and anthrax-related diseases. The remaining thirty-five studies ranged between two and fifteen syndromes with five to eight the most common (Figure 4). Among the studies with multiple syndromes, respiratory and gastrointestinal were the most common. Many also included fever, along with non-traumatic neurologic conditions, especially paralysis, rash/dermatologic conditions and undifferentiated fever.

Table 4: Number of Pre-diagnostic classifications and chief complaints

	Freq.	**Percent**	**Mean**	**SD**	**Min**	**Max**	**25%**	**75%**
Chief Complaints	55		-----	-----	-----	-----	-----	-----
with limits (% of chief complaints)	40	72.7%	3.9	6.3	1	30	1	3
Pre-diagnostic Classifications / Syndromes	73		4.0	3.9	1	15	1	7
all Chief Complaints	55	75.3%	3.6	3.9	1	15	1	6
With limits	40	54.8%	2.0	2.6	1	13	1	1
With no limits	15	20.6%	8.1	2.9	4	15	6	10

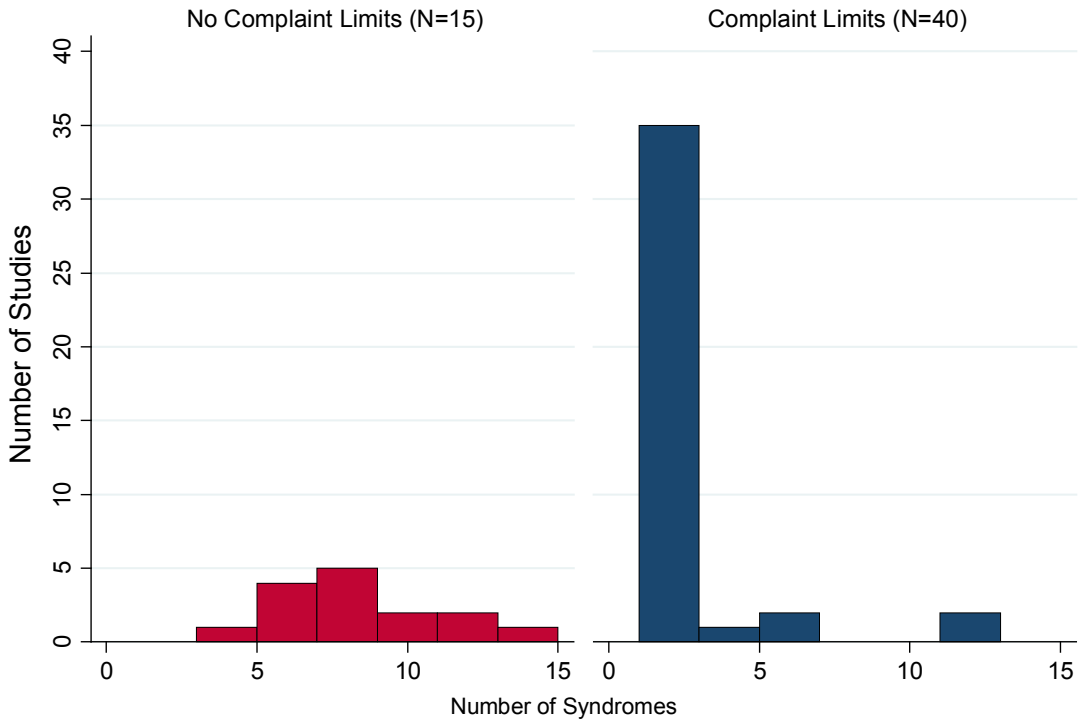

Figure 3: Number of syndromes per study, by complaint limits

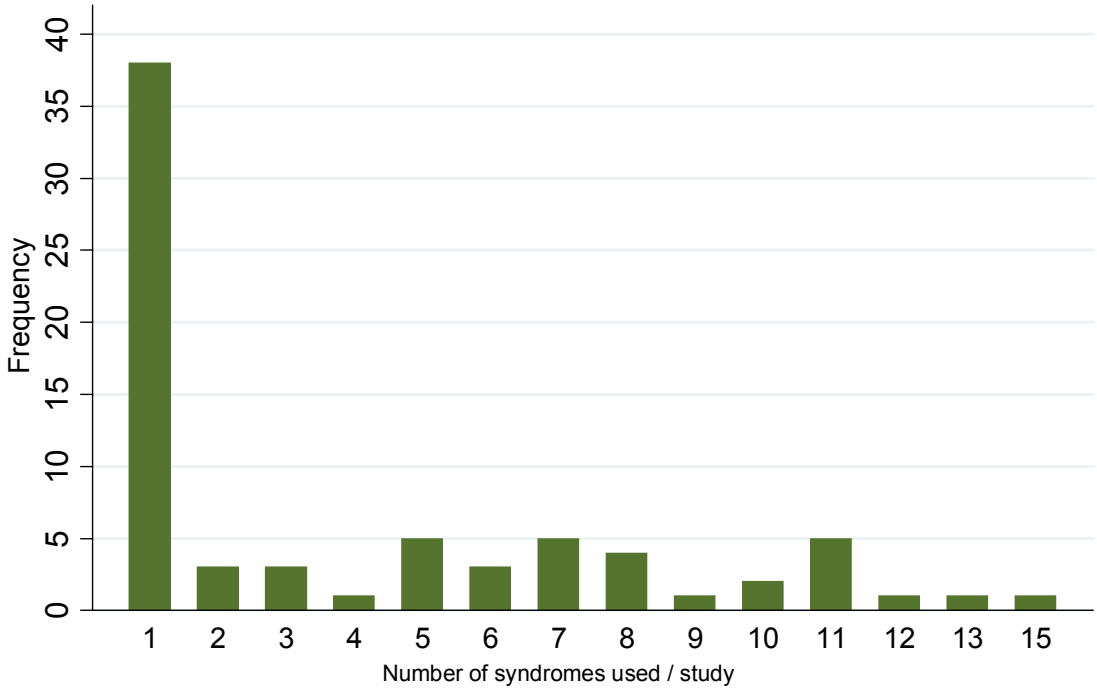

Figure 4: Number of pre diagnostic / syndromic classifications used for public health surveillance (N=73)

Systematic approaches to pre-diagnostic or syndrome classification included coding methods. The primary coding methods included were as follows;

- Clinical codes, based on clinical examination findings such as chief complaint, body temperature, blood pressure and presenting symptoms. Several types were reported, most commonly were methods from the Center For Disease Control.
- Clinical diagnoses through International Classification of Diseases, 9th revision – Clinical Modifications (ICD-9-CM).
- Laboratory codes, such as classification of microbial culture results
- Other coding methods such as pharmaceutical classes, poisonous substances classes or coding for absenteeism.

Sixty-seven studies defined classification codes for pre-diagnostic methods, thirty-eight used a single coding method; clinical (20 studies), ICD-9-CM (9 studies), Other (8 studies) or Laboratory (1 study) (Figure 5). The most common combination of coding was clinical with ICD-9-CM (16 studies) followed by clinical with laboratory (5 studies).

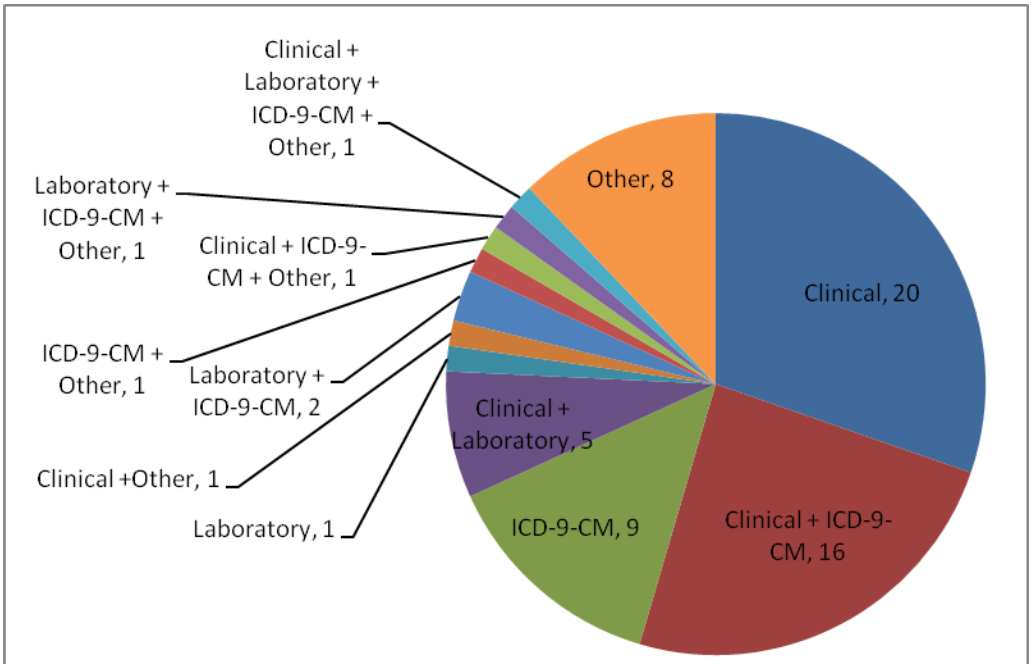

Figure 5: Studies with classification codes, N=66

2.3.4 Analysis Components, Analytical methods and Reported values

Analysis in syndromic surveillance was categorized into four components, including if more sophisticated methods of analysis were employed (Figure 6);

- Temporal methods to monitor changes in syndromes over time.
- Spatial methods to monitor changes in syndromes over a geographic region.
- Classification methods to estimate the most accurate and sensitive pre-diagnostic or syndromic categories.
- Aberration detection methods to estimate significant signals that may be generated by surveillance data.

Validation methods represented a fifth component of analysis and were used to estimate the effectiveness of the syndromic surveillance methods when compared to other pre-diagnostic methods or to more traditional methods.

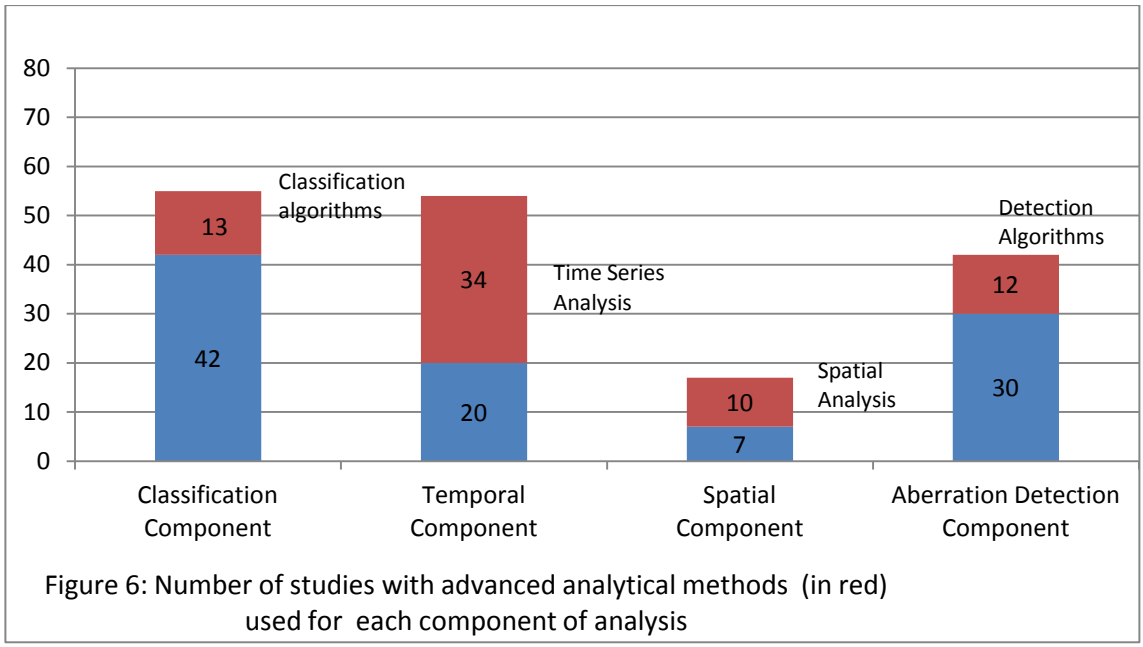

Figure 6: Number of studies with advanced analytical methods (in red) used for each component of analysis

The use of components of analysis across all studies was compared, including whether a validation method was applied or not (Figure 7a and 7b). A large number of studies focused primarily on the definition and comparison of syndromes or pre-diagnostic groups; twenty-two of fifty-five studies had only a classification component. Specific classification algorithms were utilized in thirteen of the fifty-five studies with a classification component. The most common algorithm was a naive Bayesian free text classifier that primarily grouped free text chief complaints. Time to detection compared to traditional methods and detection of significant events over time were significant components of syndromic surveillance. Temporal

components were therefore common across all studies and were also more likely to include detailed analysis (Figure 6). Time series analysis using cumulative sum control chart (CUSUM), auto regressive integrated moving average (ARIMA) and exponentially weighted moving average (EWMA) were the most common. Aberration components were primarily used to determine significant signals found in temporal or spatial analysis. These components were primarily included with three other key groups; temporal, classification/temporal, and spatial/temporal.

Spatial components occurred in large-scale surveillance systems that involved multiple data sources in an urban center or larger geographic area. These larger systems were also more likely to include more in-depth analysis due to both the larger number of observations and likelihood of receiving multiple data types (Besculides et al, 2005; Burkom et al, 2004; Carrico and Goss, 2005; Das et al, 2003; Heffernan et al, 2004; Hogan et al, 2007; Kleinman and Abrams, 2008; Kulldorff et al, 2007; Steiner-Sichel et al, 2004; Wu et al, 2008). The spatial scan statistic was the common advanced analytical method used for spatial analysis. Validation methods were used in conjunction with both classification methods for comparing pre-diagnostic groups and temporal methods for determining effectiveness of the early warning component.

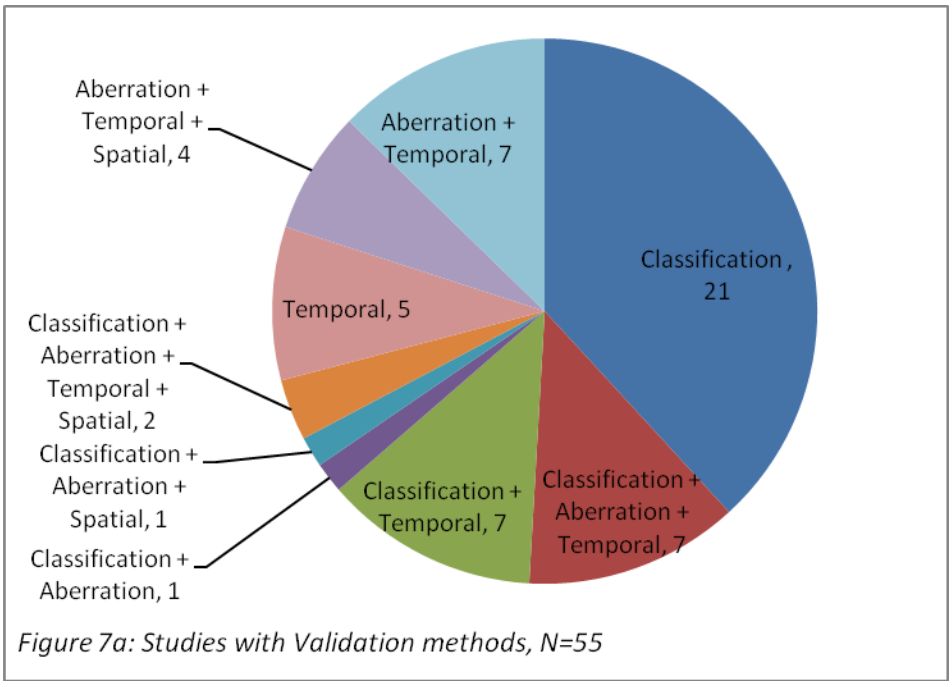

Figure 7a: Studies with Validation methods, N=55

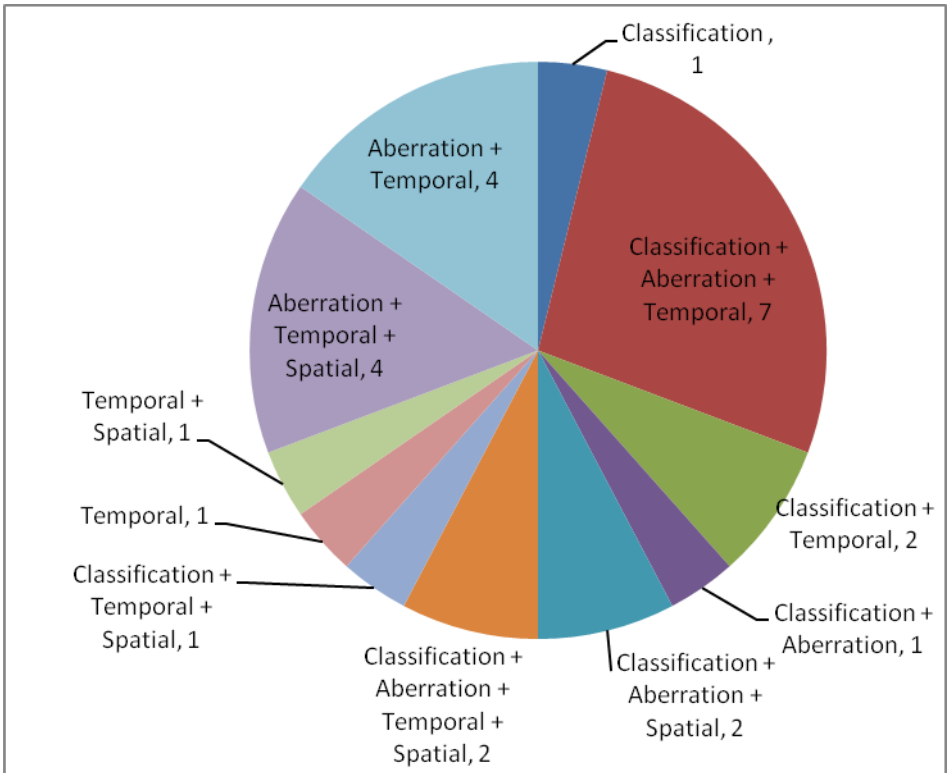

Figure 7b: Studies with no validation methods, N=26

Regression analysis, including fixed models such as Poisson, and mixed models such generalized linear mixed models (GLMM) was used frequently in comparisons between atypical pre-diagnostic groups, such as absenteeism, prescriptions, telemedicine calls and more traditional data sources such as clinical diagnoses and laboratory results (Cooper et al, 2009; Das et al, 2005; Ohkusa et al, 2005; Pattie et al, 2009; van den Wijngaard et al, 2008). Regression analysis was also utilized in several simulation studies that evaluated various monitoring tools and methods (Burkom et al, 2005; Kleinman and Abrams, 2006; Kleinman and Abrams, 2008). Twenty of the eighty-one studies used regression analysis as part of the analytical process applied to the surveillance methods.

Sensitivity, specificity, positive predictive values (PPV) were considered key measurements of statistical validity and accuracy for public health surveillance (Buehler et al). These values were the most common results reported from statistical analysis (Figure 8). Measurements of precision and agreement were also common when studies included a classification and

validation component. Correlation coefficient and kappa statistics were the most common and were used to compare syndrome classification methods in seventeen studies. Other agreement results reported included Cochrane's Q statistic. Several studies also reported probabilities, odds ratios or risk ratios when comparing the likelihood of individuals with a specific syndrome having a disease to a base population without the syndrome.

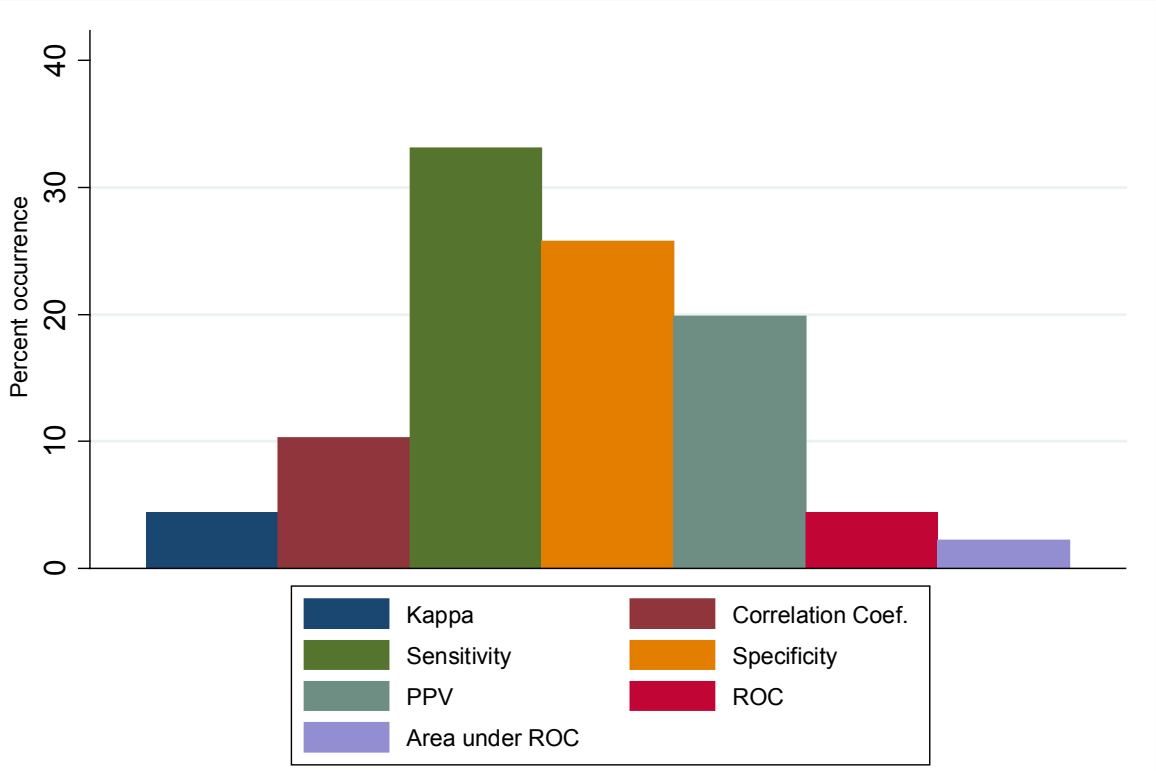

Figure 8: Measures of accuracy and agreement for prediagnostic surveillance

Reporting of sensitivity was also compared to the different analytical components; 55% with a classification component 48% of studies with a temporal component, 47% with a spatial component and 52% with an aberration detection component.

Analysis conducted for temporal purposes produced a variety of different results especially when in combination with aberration detection. The common time series analysis results reported were, the time interval to detection (time from above base line to a significant signal), the more sophisticated time to detection rate, the number of cases that had exceeded a set standard deviation (typically two) from the mean and CUSUM values, such as z scores.

2.4 Discussion

Systematic reviews of public health surveillance have identified the capacity of surveillance systems to utilize information from a broad public scope collected routinely for administrative, business or case management needs and stored electronically (Bravata et al, 2004; Leal and Laupland, 2008). The systems provide a potentially inexpensive and efficient means of collecting and collating data from multiple sources and types, covering large geographic areas and often in real time or near real time. In order to effectively use these systems, the information must provide adequate sensitivity and specificity compared to traditional surveillance methods and should meet or improve upon the timeliness of disease detection. The need for sensitivity, specificity and timeliness define two key challenges; first the data have been collected for other purposes and does not readily transfer to a sensitive indicator of a specific disease or group of diseases. Information from either within a data type or across data types must be classified into sensitive pre-diagnostic or syndromic indicators to accurately detect the disease or condition under surveillance. Second, even once a sensitive indicator has been developed, the amount and non-specific nature of data results in a significant number of trend outputs and variations that needs to be balanced between sensitivity, specificity and timeliness. If the level of detection is set too high, not only will the sensitivity of the system decrease, but the timeliness of detection may not be sufficient to provide rapid enough indications. Initial reviews of these systems identified a lack of reference standards for classification, a lack of comparison even between traditional surveillance methods to determine timeliness, an inadequate evaluation of data for sensitivity and specificity, and significant challenge in distinguishing between statistical anomalies and significant public health events (Bravata et al, 2004; Hurt-Mullen and Coberly, 2005). Additionally, many systems had incorporated spatial data without evaluating whether a combination of temporal and spatial data provided an improvement over temporal data alone (Bravata et al, 2004). In spite of the initial gaps in standards and in effectiveness, passive electronic surveillance systems using pre-diagnostic/syndromic methods have become well established in public health surveillance and are considered to be practical tools for epidemiologists in the rapid detection of emerging, infectious and bioterrorism related diseases (Hurt-Mullen and Coberly, 2005; Leal and Laupland, 2008).

The overall results of this review indicate that syndromic surveillance in public health has focused primarily on rapid detection from front line medical contact at emergency departments or outpatient clinics. However, non-traditional data types including pharmacy, telemedicine, absenteeism, ambulance dispatch and others were explored in thirty-three of the studies reviewed. While recognized as potential data for syndromic surveillance, laboratory data were primarily utilized for comparison and validation. Only one study evaluated laboratory submission data as an pre-diagnostic indicator (Hoabo et al, 2005). The results support the tendency to utilize multiple data sources for broad area coverage and improved timeliness, but the inclusion of multiple data types in the evaluations was not as common, even if the system under evaluation included additional data types. The key disease groups of interest to public health surveillance, infectious diseases, emerging disease and bioterrorism events, were reflected in the studies reviewed.

Classification of pre-diagnostic or syndromic indicators was found to be a critical part of syndromic surveillance. When studies focused singularly on classification, it was primarily to develop and validate a specific syndrome or syndrome group; of the fifty-five studies with classification components, twenty-one did not have any additional components other than validation. The initial findings at point of contact, especially chief complaints, were the key components of pre-diagnostic classification. ICD-9-CM codes appeared to contribute significantly to classification methods, either as part of classification itself or as a part of a validation. The use of more advanced techniques to classify syndromes was not as well developed and focuses specifically on free text chief complaints. While the use of language processors and support vector machines was noted, ten of the thirteen studies that described a sophisticated syndrome classification method used the same one; a naive Bayesian classifier utilized by the Real Time Outbreak Detection System laboratory at the University of Pittsburgh (Chapman et al, 2004; Chapman et al, 2005a; Chapman et al, 2005b; Ivanov et al, 2002; Ivanov et al, 2003; Mikosz et al, 2004; Moore et al, 2008; Muscatello et al, 2005; Reis and Mandl, 2004; Wagner et al, 2004).

The number of syndromes under evaluation remained very low in this review, typically only one. The most obvious reason is a focused approach to evaluation where estimating sensitivity, specificity and timeliness on many syndromes may be very difficult to complete in a

single research study. However, from a public health perspective single syndromes with a respiratory basis are very important and warrant a strong focus: many of the most significant disease include significant respiratory components; influenza, SARs, and anthrax used for bioterrorism (Bravata et al, 2004; Chapman et al, 2005a; Ritzwoller et al, 2005). Additionally, while less common but with potential application to veterinary medicine, single syndromes were utilized for focused identification of specific diseases in areas where laboratory confirmation is not readily available (Aggarwal and Kumar, 2004; Mathews et al, 1998; Yin et al, 2008). The directed approach was also consistent in limits placed on the chief complaints captured for syndrome classification. When no limits on chief complaints were placed, the number of syndromes increased from a mean of two to a mean of eight. The relationship between chief complaints and syndromes is causal only in that limits on chief complaints appear to be placed when the number of syndrome is limited *a priori*. More importantly, this review suggests that if syndromic surveillance is to include multiple syndromes, the information necessary to classify those syndromes should be as broad as possible.

Considerable emphasis was placed on the use of time series analysis and aberration detection. As noted above, there is a great need to distinguish between statistically anomalies and significant public health events. Specific detection algorithms such as What's Strange About Recent Events (WSARE) and the Bayesian Aerosol Release Detector (BARD) have been developed to address these concerns (Buckeridge et al, 2005; Hogan et al, 2007; Kaufman et al, 2007). Additionally, the inclusion of spatial analysis has attempted to provide more refined signals (Kulldorff et al, 2005; Kulldorff et al, 2007). The need for advanced analysis comes in part because of the large numbers of selected cases and associated case data that occurs in public health surveillance. When case numbers were reported in the studies reviewed, selected cases often exceeded 60,000 for a time period of one year or less. Multiyear studies exceeded 400,000 cases with the largest, a nationwide pharmacy surveillance system reporting 2.6 million. The capacity to determine if a significant public health event has occurred through case follow-up may be limited by such high case numbers (Buckeridge et al, 2005; Hurt-Mullen and Coberly, 2005; Terry et al, 2004). Additionally, non-traditional data sources and passive surveillance can be affected by weekly, seasonal and onetime events that may create signals that are difficult to distinguish from significant public health events (Carrico and Goss, 2005). Response and validation of a significant public health event from syndromic surveillance must

include additional levels of data analysis for effective response. In veterinary medicine, the greater issue would likely be a lack of case numbers to generate sufficient baseline. However, sophisticated temporal and spatial analysis has been applied in veterinary surveillance (Odoi et al, 2009).

Evaluation of syndromic surveillance has developed sufficiently to include the generally excepted measures of accuracy for public health surveillance. Sensitivity was reported in forty-five of the eighty-one studies, represented greater than 30% of all results reported and was indicative of method validation. Additional accuracy results, such as specificity, PPV, and area under ROC were also included with sensitivity in some studies. Measures of agreement (kappa or correlation coefficients) with syndrome classification demonstrated greater evaluation of effective syndromes. Timeliness, sensitivity and specificity of syndromic surveillance has reportedly improved by utilization of multiple data sources and data types (Hurt-Mullen and Coberly, 2005; Ritzwoller et al, 2005). However, in this review studies with multiple data sources did not routinely include sensitivity as a result. Intuitively, multiple data sources, especially of the same type would seem to give better surveillance over space and time. Some of the difficulty comes from the rarity of events such as bioterrorism (Bravata et al, 2004; Buckeridge et al, 2005), but overall there is a need in public health for large multi data source and type surveillance systems to report sensitivities.

Syndromic or pre-diagnostic surveillance has been attempted in some areas of veterinary medicine (Bartlett et al, 2010; Shaffer et al, 2008; Van Metre et al, 2009) but has not been explored to the same degree as is the case in public health. This review has highlighted challenges and advantages to the application of syndromic surveillance in animal health. Databases containing chief complaints, clinic examinations and clinical diagnoses are not readily available in animal health. Globally, systems involving animal hospital based pre-diagnostic surveillance have been implemented, but there has been no substantive evaluation of their sensitivity, specificity and timeliness. Laboratory databases are more available, but are often limited to a specific region or do not represent all of the animal population in a given area (e.g. laboratory services may be limited to only one sector of the animal population). Public health syndromic surveillance has explored non-traditional data sources to further expand surveillance capacity. Similar approaches could be taken in animal health.

As in public health, animal health surveillance that utilizes syndromic methods should focus on the key disease categories that are of significant concern. Emerging disease, reportable diseases, zoonotic diseases and significant changes in endemic diseases could form the key strategic categories for any animal health syndromic surveillance system.

Syndromic surveillance in public health has made good use of globally accepted medical disease coding standards, such as the ICD-9-CM. Systematic, generally accepted disease nomenclature does not exist in veterinary medicine. Other than expert opinion, the classification of pre-diagnostic data into significant syndromic indicators will need to utilize more disparate data, rely heavily on the data source and the data itself to estimate significant syndrome classifications. The ability to provide comparative data for the measurements of accuracy and agreement assist in developing informative classifications. For example, laboratory data can provide pathological diagnoses and test results to compare to classification of submission information.

Population demographics may also present an additional challenge in veterinary medicine compared to public health. Large portions of domestic animal populations (i.e. food animal production) live entirely in herd/flock situations where disease contact and spread occurs primarily at the group level. Animal health events are often recorded for groups of animals and data from single individuals are interpreted at the herd level. Identifying and evaluating group level dynamics for surveillance in data collected for other purposes may be very important in animal health compared to public health.

Timeliness of animal health data will rely heavily on how quickly and practically information can be submitted. Currently, if clinic databases could be reasonably linked only companion animal medicine has the ability to supply case information that would compare to the real time approach in public health. However, some current clinic based systems approach real time with daily submission of case data. As is the case in public health, laboratory submission data in animal health may have difficulty providing sufficient timeliness (Hoabo et al, 2005; Shaffer et al, 2008). However, in regions where other sources of information for animal health surveillance are limited, laboratory data may represent a viable option to explore.

Animal health can learn from the experiences of public health surveillance and ensure that syndromic surveillance is developed in ways that can effectively measure and report sensitivity, specificity and timeliness. Such measures will be critical to adequately informing policy and responding to significant animal health events. The public health experience must be further tempered for animal health by the amount of data available, the data sources available, the population demographics and the comparable lack of diagnostic references standards. However, the opportunity to explore syndromic surveillance further in animal health is considered a legitimate approach to address large scale animal health surveillance needs. The public health experience in syndromic surveillance reviewed in this study demonstrates the systematic approach necessary to move forward in animal health. The principles of such a systematic approach that need to be applied are: a) Identify and evaluate the use of clinical, laboratory and non-traditional data sources. b) Develop meaningful syndrome classifications through methods applicable to the available data and resources. c) Determine the most appropriate signals and the associated timeliness. d) Ensure an analytical approach with appropriate measures of association and validation to avoid false interpretations while capturing critical early signals.

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Chapter 3: Exploration and evaluation of pre-diagnostic data from a regional animal health laboratory for the purpose of syndromic surveillance in swine

3.1. Introduction

Syndromic surveillance methods enhance traditional early warning surveillance by analyzing continuously acquired pre-diagnostic data for earlier detection and response of disease events (Dorea et al, 2013; Hiller et al, 2013; Hoinville et al, 2013; Katz et al, 2011). The use of laboratory pre-diagnostic data, such as test requests and specimen types included in case submissions, has been identified as a viable approach for syndromic surveillance in human and animal health (Dorea et al, 2012; Dorea et al, 2013; Ma et al, 2005; Odoi et al, 2009; Shaffer et al, 2008). Reviews of syndromic surveillance methods and systems have identified that effective analysis and evaluation of data sources are essential to establish appropriate syndrome definitions for syndrome classification, to determine the availability of continuously acquired baseline variables for timely aberration detection and to determine availability of diagnostic outcomes for validation in the form of syndrome sensitivity and aberration detection performance (Buehler et al, 2004; Dorea et al, 2011; Katz et al, 2011). The evaluation of a syndromic surveillance data source is important for several key reasons: As syndromic surveillance relies on information collected for other purposes, it is essential to understand the reasons for the data collection so that the relevance to the health and welfare of the source populations can be estimated. Additionally, the type of data, the structure and the applied data standards are important to determine what pre-diagnostic data are available for syndrome classification and for estimating syndrome sensitivity. Finally, determining the timeliness of the data, what the underlying trends are and what baselines can be established, are essential in understanding the capability for anomaly detection and accuracy. These key reasons are especially true for animal health data sources (including laboratories) where there are species differences, inherent multilevel clustering of certain animal populations (herds and flocks in food production), non-diagnostic health assessments, less complete data capture, less developed data standards and less overall timeliness in reporting (Amezcua et al, 2013; Dorea et al, 2011; Dupuy et al, 2013a; Kosmider et al, 2011).

To estimate the relevance of pre-diagnostic laboratory data in animal health syndromic surveillance, it is important to understand the reasons for submissions (Dorea et al, 2011; Gibbens et al, 2008). Reasons for submissions to animal health laboratories are impacted by

submission bias similar to other animal health data sources, where the cases submitted may not fully represent the health or demographics of the source populations. Submission bias is a form of selection bias where the choice by submitting veterinarians to submit samples is affected by reasons other than disease investigations, such as economic factors (e.g. feed prices, currency exchange rates, carcass values), requests for non-diagnostic testing (e.g. international trade, vaccine effectiveness, regular health monitoring, research) and professional experience (Bartlett et al, 2010; Dohoo et al. 2009). When significant disease issues are presented in swine herds and other animal populations, it has been demonstrated that veterinarians will increase diagnostic pathology submissions to better understand the disease processes (Gibbens et al, 2008; O'Sullivan et al, 2012; O'Toole, 2010). However, evaluating data sources for an understanding of what factors may be contributing to submission bias is helpful in determining the overall effectiveness of detecting significant disease events in animal populations using syndromic methods. As noted above, this is especially true for food animal production where many factors may contribute to submission bias.

To understand how pre-diagnostic data from animal health laboratories may be classified into relevant syndromes, it is important to know what data are available and how it is coded. Many laboratory submissions and records do not contain or do not have ready access to all of the information in a recommended minimum data set proposed by Kloeze (Kloeze et al, 2012). Specifically, disease classification by submitters (clinical diagnoses) and/or reasons for submissions are not consistently provided or recorded. Additionally, not all information is coded in a format that would provide a more efficient means of classifying data into syndromes (Dorea et al, 2011; Gibbens et al, 2008; Shaffer et al, 2008). Data coded systematically using standardized definitions provides a means of mapping data clusters for syndrome classification or of grouping complex diagnostic outcomes for validation in syndromic surveillance(Dorea et al, 2011; Gibbens et al, 2008). Standardized disease nomenclatures such as the Logical observation identifiers names and codes (LOINC) used in human health laboratories provide both well established data definitions and a means to compare data across different sources using the same codes (Ma et al, 2005; Sintchenko and Gallego, 2009). However, as long as the coding system is defined with established rules for use, a data source that has its own internal coding system for diagnostic data has been demonstrated to provide, at minimum semi –

structured data for classification and validation (Gibbens et al, 2008; Hyder et al, 2011; Shaffer et al, 2008).

Validation of syndrome classification and detection algorithms has been identified as an important step in both clinical and laboratory syndromic surveillance methods as it establishes the sensitivity, specificity and diagnostic performance of the surveillance components (Dorea et al, 2012; Guasticchi et al, 2009; Kashiouris et al, 2013; Leal and Laupland, 2008). Furthermore, it has been recognized that validation methods, if applied routinely through automated or manual means, may be used to keep syndromic surveillance components up to date (Dorea et al, 2013). Syndromic surveillance systems in public and animal health have achieved both of these steps by linking the surveillance data to objective outcome data, such as laboratory results and to professional assessments, such as pathology diagnoses (Amezcua et al, 2013; Hiller et al, 2013; Leal and Laupland, 2008; Shaffer et al, 2008). Sensitivities and positive predictive values for syndrome classification compared to diagnostic outcomes have been utilized frequently in public health to ensure the classifications are representative of the targeted heath conditions. When possible, this "gold standard" approach is seen as a preferred option for syndrome validation (Guasticchi et al, 2009; Hiller et al, 2013; Kleinman and Abrams, 2008; van den Wijngaard et al, 2008). Diagnostic outcomes are easier to use as "gold standards" in public health data sources because the common use of standardized disease nomenclature to assign case outcomes. In veterinary medicine, diagnostic outcomes are also a preferred method of validating syndrome classification. However, the semi structured nature of diagnostic outcomes in many animal health data sources require more complex analyses and/or further professional assessment to provide validation methods for syndrome classification (Dorea et al, 2011; Dorea et al, 2013).

The purpose of this chapter is to analyze and evaluate the data available from a regional animal health laboratory for the larger intent of enhancing its contribution to early warning surveillance in the regional swine population by incorporating syndromic surveillance methods. Specifically; (a) to describe the type of submission information and the underlying trends in typical swine cases submitted to a regional animal health laboratory (b) to describe the types of specimens submitted, the diagnostic procedures requested and the outcomes of the typical swine cases, (c) to determine the availability and type of test requests and submitted specimens for use in syndrome classification for detection of significant swine health events

and (d) to conduct an analysis of laboratory results and pathology diagnoses that establishes diagnostic outcomes that may be used for validating syndrome classification.

3.2. Methods

3.2.1 Data Source

Veterinary Diagnostic Services (VDS) is a full service animal health diagnostic laboratory in the province of Manitoba, Canada and is a component of the Agri-Industry Development and Innovation branch of the provincial department of Agriculture, Food and Rural Development (MAFRD). VDS fulfils the primary animal health laboratory role of providing diagnostic and pathology services to practising veterinarians and their clients. Additionally, VDS actively contributes to the surveillance and identification of emerging diseases through the traditional means described by O'Toole, especially for diseases such as porcine circovirus (PCV2) and porcine epidemic diarrhea (PED) (O'Toole, 2010). Finally VDS is a full participant in two key animal health networks for disease surveillance and response; the Canadian Animal Health Surveillance Network (CAHSN) and the Canadian Animal Health Laboratory Network (CAHLN). As part of CAHSN, VDS is one of the network's participating laboratories that supplies case submission data on a daily basis to a national animal health surveillance and disease response initiative (Kloeze et al, 2010). The CAHLN involvement commits VDS to work with other Canadian animal health laboratories to improve standard approaches for conducting and reporting test results, as well as improved standardization of disease coding.

VDS was estimated to receive 75 to 80% of all porcine laboratory submissions from the province. The percentage of diagnostic pathology cases was estimated to be higher due to a centralized location, readily available expertise and the specific public funding of food animal laboratory submissions. The laboratory infrastructure and operations was centred in Winnipeg, the largest urban centre in Manitoba. Winnipeg is a regional commercial and transportation hub within close proximity to the majority of Manitoba swine production. As a regional animal health laboratory, VDS maintained a high degree of in-house professional expertise and technical capacity in general necropsy and histopathology, diagnostic virology and microbiology. This resource allowed for the interdisciplinary approach to significant regional animal health events promoted by O'Toole, creating a close collaboration with clinical veterinarians which in turn encouraged diagnostic and pathology submissions (O'Toole, 2010).

Public funding to VDS provided 70% of the financial costs for all diagnostic testing, non-diagnostic testing and pathology expertise for submissions from Manitoba livestock herds and poultry flocks. The contribution of public funds to laboratory infrastructure and operations, in association with data sharing agreements provides VDS the opportunity to effectively share laboratory data for early warning surveillance.

Swine submissions to an animal health laboratory can be categorized based on clinical demands: to conduct herd level monitoring of endemic diseases or hazards (e.g. antimicrobial resistance), to implement and adjust herd based risk mitigation procedures for endemic diseases (e.g. vaccine effectiveness), to confirm freedom from disease for trade purposes and to establish a diagnosis in an animal health event. The differentiation between the types of submissions to animal health laboratories for swine health is important. Establishing diagnoses for clinical disease often involves the direct intervention of veterinary pathologists. Cases that are submitted for these reasons inherently imply that a change of health status has occurred in the affected animal or herd. Herd level monitoring for risk mitigation or freedom from disease is typically done to confirm health status has changed.

VDS utilized a Laboratory Information Management System (LIMS) developed and maintained by in-house technical staff. The LIMS is primarily designed to maintain client information and laboratory results for reporting and billing purposes. The system has limited data retrieval capabilities that allow for static queries of the database for other purposes, such as surveillance. As with other veterinary diagnostic laboratories (Dorea et al, 2012; Gibbens et al, 2008; Shaffer et al, 2008); (a) a common *case number* is given to all samples submitted and tests performed from an individual health event from the same location submitted on the same day. (b) The *case number* more accurately reflects a submission number; submissions may represent single animal health events (or cases). However multiple submissions on multiple days from the same animal health event (even at the same location) will generate different case numbers. The samples submitted and the test requests for each submission are recorded into the LIMS on the day received. The key variables collected and recorded routinely across all submissions to VDS are in table 1. Diagnoses and Organ system classifications (Table 2) are standardized and used within the LIMS as in other diagnostic laboratories (Dorea et al, 2012; Gibbens et al, 2008; Shaffer et al, 2008). However, like many other animal diagnostic

laboratories, general standardized coding systems, such as SNOMED, LOINC or HL7 are not used (Dorea et al, 2011). For diagnostic pathology submissions, the case diagnoses were represented through diagnoses and organ systems codes, assigned in order of significance; a primary diagnosis may be followed by up to 12 additional diagnoses.

Table 1: Data Fields collected for laboratory submissions to Veterinary Diagnostic Services Laboratory Information Management System (LIMS)

Submission Field	Description
Case number	Submission number from a single event at a location on a given date
Species	
Submission Date	
Risk Group	Total group size, number at risk, number sick and number dead
Problem duration	Duration of health event, in days or months
Practitioner Identification	Unique Practitioner and clinic identifiers, which include postal code and nearest city.[1]
Producer Identification	Unique producer identifiers including postal code and nearest city.[1]
Submitted Animals	Number of dead and/or live animals submitted for necropsy
Individual animal information	Gender, weight and age. Routinely collected for companion animal cases. Not routinely provided for livestock cases
Specimens submitted	Description and code for all specimens submitted with a case
Test requests	Description for all tests requested for a case, including individual test and laboratory codes
Diagnostic Codes	Fields for diagnostic pathology submissions, in order of most significant findings: 12 possible fields, 2500 coded diagnoses
Organ System Classification	Organ system associated with pathology diagnoses, 12 possible fields. 18 organ system descriptions[2]
Test Results	Results for each test conducted per submission. Values and units specific to each test procedure, additional field for further comments

1. Premises identification numbers for land locations associated with veterinary clinics and livestock operations are now mandatory on VDS' LIMS. They were not mandatory at the time the data were extracted. Postal codes and nearest city were the only available geo-locations.
2. Organ systems and associated codes are described in Table 2.

Table 2: 18 organ systems available for assignment to pathology submissions

Code	Organ System	Code	Organ System
1001	Bones /Joints/ Synovial tissue	1010	Male Genital
1002	Cardiovascular	1011	Multiple Systems
1003	Eye /Ear/ Sensory	1012	Muscle
1004	Endocrine	1013	Nervous
1005	Female Genital	1014	Respiratory
1006	Hematopoietic	1015	Skin and Appendage
1007	Lower Digestive	1016	Unknown
1008	Lymphoreticular	1017	Upper Digestive
1009	Liver /Bile /Pancreas	1018	Urinary

All available data from all species were extracted in a single query using the available query platform from January 1st 2003 to March 15th, 2009. January 1st 2003 was used as a cut off because it represented the start of the first full year on the LIMS system that was developed in-house. Prior to that date, VDS had used commercial LIMS software.

3.2.2 Data Evaluation

Collation and analysis of laboratory data were attempted following the principles of a minimum data set for surveillance purposes (Kloeze et al, 2012). The minimum data set was used to define the essential, core elements of the data from the LIMS. The data were screened and evaluated using statistical software(*Stata Statistical Software: Release 11, 2009*). Similar to the method described by Gibbens et al, the porcine submissions were divided into those submitted for pathology work up (Pathology submissions) and those submitted for limited specified testing (Non-pathology submissions)(Gibbens et al, 2008). However, unlike the system described by Gibbens, the divisions were made based on veterinary practitioner requests and not on reasons for submissions; reasons for submissions were not in the data base because they are not directly captured (or coded) in the submission process. Instead, these reasons are part of additional text comments provided by the practitioner. Additional text comments were not captured in the extract and would have required text mining methods to extract information.

Pathology submissions included tissues (from field necropsies) or full carcasses from an animal health event. The submitting veterinarian had requested a full pathology work up and had included specific test requests for suspect diseases. These submissions were diagnostic cases and the pathology work up concluded with a series of pathology diagnoses assigned to organ systems and in order of relevance. The data evaluation summarized the common diagnoses and their classification by organ system. It was recognized that these submissions may be affected by misclassification bias in that practitioners may not submit the right specimens. Such misclassification bias would be indicated in pathologists' case comments, but these comments were not available in the extract provided. Non-pathology submissions were also submitted through veterinarians, but were submitted for diagnostic or non-diagnostic reasons. As noted above, reasons for submission were not coded and were not available in the extract provided. Pathologists' reviewed all testing completed at VDS and provided

comments on the final reports. However, these comments were not pathology diagnoses and were captured only in the comment fields.

The distributions of specimens submitted and tests requested were determined for both pathology and non-pathology submissions. The intent was to describe the variety of specimen types and test requests available as pre-diagnostic indicators for potential syndrome development, especially for pathology submissions. To provide groupings of pathology outcomes for syndrome validation, pathology submissions were further evaluated to determine the frequency and distribution of specific diagnostic codes and organ systems across diagnostic variables. The frequencies and distributions were used to estimate the extent additional diagnoses were assigned to pathology submissions. The distributions across diagnostic categories were also used to determine the most frequently assigned organ systems and the most frequently assigned diagnostic codes. The most frequently assigned organ systems were cross referenced with the most common diagnostic codes to compare frequent codes with frequent organ systems in the primary diagnosis category. A single "Other" organ systems group was used for grouping less frequently assigned organ systems and the diagnostic codes were compared with this group. In order to further assess the viability of using test requests and specimen types for syndromic development, the distributions of specimen types and test requests across organ system groups were also determined. For non-pathology submissions, the evaluation was limited to the frequency and distribution of test requests and specimen types.

3.2.3 Data Analysis

As noted above, the outcomes for pathology submissions may be used to form the basis of a validation for syndrome classification. Since each pathology diagnosis is assigned a diagnostic code and an organ system category in order of priority, a simple approach may have been to group submissions on their primary diagnoses. However, in order to have the most representative outcomes for validation, it is necessary to evaluate if additional diagnoses (outcomes) for each submission would impact the grouping of submissions into outcome variables. Pathology submissions were evaluated for clustering across the primary and additional diagnoses using Multiple/Joint Correspondence Analysis (MCA) and ascending hierarchical cluster analyses. MCA is a form of Multiple Factor Analysis (MFA), where, like

other forms of MFA, the mathematical theories of linear algebra are used to identify geometric associations between variables (Blasius et al, 2009; Le Roux and Rouanet 2010a). MCA is a specific analysis for categorical variables with more than a binary outcome and is often used in social sciences to describe relationship patterns among individual answers to questionnaires with multiple categories of potential responses. The analysis generates coordinates for observations on a series of dimensions (axes) that are each weighted by the frequencies of the categories in each variable (principal inertia). The coordinates form a cloud of points based on dimensionality where each dimension is represented by principal axes (1,2,3, etc) ranked in decreasing order of category contribution to the overall inertia: the first axis has the groups of categories with the highest percentage of contribution to inertia, the second axis the next highest, and so on. Each dimension describes variation in clusters of observations within categories not described by the previous axis, thereby identifying differences in variation contributed by different category groups (Le Roux and Rouanet 2010b). Eigenvalues are the measurements of variance between coordinates, the average variation for each dimension is represented by mean eigenvalue for that dimension (axis) and the sum of all eigenvalues is the total variation in the cloud (Le Roux and Rouanet 2010c). Ascending hierarchical clustering applied to a cloud of points within the variance criterion is considered the companion method of MCA, that identifies "nested partitions" (in this situation, groups of cases) within the cloud (Le Roux & Rouanet 2010a). The hierarchical clustering method uses the coordinates described through MCA and places the associated observations into "new" outcome variables that take the dimensions and their percentage variance contribution into account. Components of MFA and hierarchical cluster analysis have been used to define and evaluate animal health syndromic surveillance methods in abattoirs and wildlife pathology (Dupuy et al, 2013b; Warns-Petit et al, 2010).

To evaluate the impact of pathologists assigning multiple additional diagnoses to pathology submission outcomes, clusters of diagnoses were evaluated using MCA on submissions with up to 2 additional diagnoses or up to 4 additional diagnoses. When submissions did not have any additional diagnoses, they were assigned as "missing". Two approaches to conducting MCA on pathology diagnoses outcomes were considered, both using frequently assigned organ system categories, including the "Other" category, from the data evaluation: In the first method, additional diagnoses were included only if an organ systems category was assigned. In the

second method, all additional diagnoses were included, with "missing" treated as an additional category. For the second method, the primary diagnosis would still have only one of the frequently assigned organ system categories as there was never a "missing" primary diagnosis. However, each additional diagnosis could have one additional outcome, "missing" if there were no further diagnoses identified by the pathologists. Coordinates for submissions were obtained for three dimensions using standardized or principal normalization, based on the percent contribution to principal inertia (Le Roux & Rouanet 2010c). The contributions of organ system categories in each dimension were described from the MCA. Cluster analysis on the submissions, using the MCA coordinates, was completed using Ward's hierarchical agglomerative linkage method (Le Roux & Rouanet 2010c; Stata Press 2009). Cluster analysis was conducted using coordinates from the principal inertias across three dimensions. The statistical software weighted the coordinates based row scores (inertias from organ system categories) and dimension scores (inertias from diagnoses categories). If missing values were included (by including the "missing" category), cluster analysis was conducted on both row and dimension scores. An alternative approach to missing values was to exclude them from the analysis . Cluster analysis was conducted on row scores only, since excluding the "missing" category excluded dimension scores from submissions without additional diagnosis.

3.3 Results

3.3.1 Overall Submissions

VDS' LIMS system data set had a total of 115,108 cases involving 906,184 specimen/test result combinations for the 2003-2009 time period (approximately 8 specimen/test per case). For each submission the tests were coded into one of 301 possibilities and the specimens were coded into one of 347 possibilities. Twenty one thousand six hundred and sixty five (21665) porcine cases were extracted from data set, representing 19% of the total case submissions. The porcine submissions included 492,775 specimen/test result combinations, 54% of the total specimen/test result combinations submitted (approximately 23 specimen/test results per case). There was an average of 3494 porcine cases per year; with 4726 (21.8 percent) pathology submissions and 16,939 (78.2 percent) non-pathology submissions (Table 3).

The extracted submission information was not complete when compared to the recommended minimum data set(Kloeze et al, 2012); Geographic location was limited to postal code. Farm

type and group type were collected but not present in the data extract. The total population of animals tested, number sick and number dead were provided by practitioners in less than 5% of the submissions. Disease classification by the practitioner was reported to be provided in case histories included with laboratory submission forms. However, case histories were not included in the syndrome categories within the LIMS because they occur as free text, making it extremely difficult and time consuming for VDS staff to enter the information . Surrogate variables such as the test(s) performed, the specimen(s) submitted and the organ system classification for pathology cases were available. The test results, the disease agent(s) identified and codified final pathology diagnoses for pathology submissions were available. Final laboratory diagnoses for non-pathology submissions were restricted to results and comment fields. No specific coding for these outcomes was utilized.

An average of 289 porcine cases were submitted per month with a standard deviation of 69.9 and a range of 126 to 433. While pathology submissions were consistent throughout the time period, non-pathology submissions varied greatly, year over year (Figure 1). The highest level of monthly submissions occurred from 2005 to 2007. Seasonal variation was observed on average submissions per week, with a greater number of submissions occurring in winter and fall. However, this variation appeared consistent year over year (Figure 2).

Table 3: Porcine submissions by year; Total submissions, submissions with pathology diagnoses and submissions with no pathology diagnosis.

	Frequency			Percent of total cases			Cum. Percent		
Year	Total	Pathology Submission	Non Pathology Submission	Total	Pathology Submission	Non Pathology Submission	Total	Pathology Submission	Non Pathology Submission
2003	2,505	679	1,826	11.56	3.13	8.43	11.56	3.13	8.43
2004	2,785	694	2,091	12.85	3.20	9.65	24.42	6.34	18.08
2005	3,703	717	2,986	17.09	3.31	13.78	41.51	9.65	31.86
2006	4,366	961	3,405	20.15	4.44	15.72	61.66	14.08	47.58
2007	4,329	933	3,396	19.98	4.31	15.68	81.64	18.39	63.25
2008	3,313	624	2,689	15.29	2.88	12.41	96.94	21.27	75.67
2009[1]	664	118	546	3.06	0.54	2.52	100.00	21.81	78.19
Totals	**21,665**	**4,726**	**16,939**	**100.00**	**21.81**	**78.19**			

1. Cases up to March 15, 2009

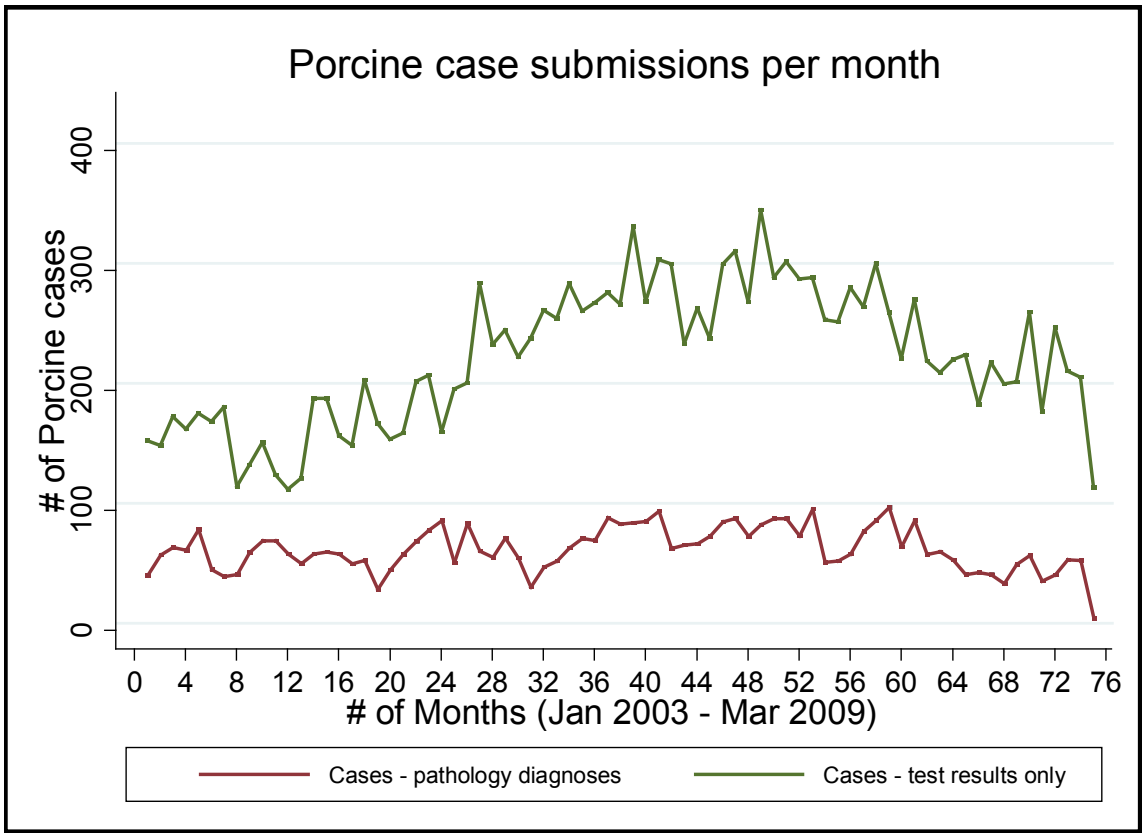


Figure 1: Monthly porcine submissions to Veterinary Diagnostic Services from January 1, 2003 to March 15, 2009.

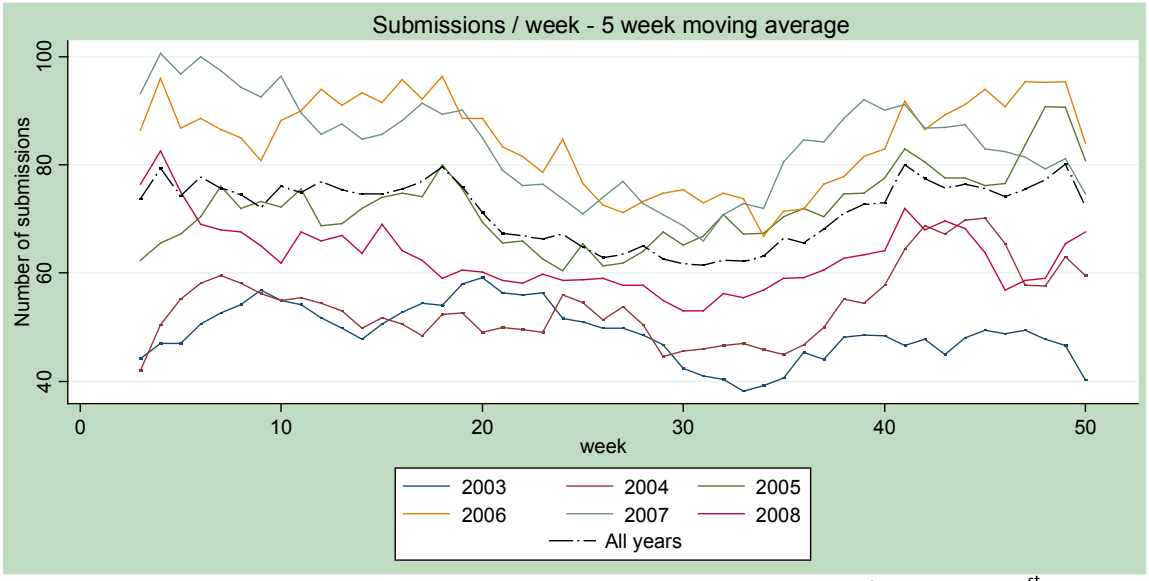


Figure 2: Weekly porcine submissions to Veterinary Diagnostic Services, by year from January 1st, 2003 to December 31st, 2008.

3.3.2 Pathology Submissions – Test requests & Specimen type distributions

Porcine pathology submissions included 65 test codes and 79 specimen codes. The most common 25 test requests accounted for greater than 95 percent of all test requests and the most common 25 specimens submitted accounted for greater than 93 percent of all specimen types submitted (Table 4). Figure 3 represents the distribution of specimens submitted and test requests for all pathology submissions. The distributions suggest a reasonable number of specimen types and tests were conducted on each case. However, predominant test requests and submitted specimens were not specific (histology, necropsy, aerobic culture, fixed tissue and carcass) and were not easily classified into syndrome categories. Appendix I represents further distributions of specimen type and test distributions by the most common organ systems groups. As with the overall distribution, there were many specimen types and test requests that are non-specific. Additionally there were several specimen types and test requests that would normally be thought of as organ system specific that were frequent in more than one organ system. For example, lung specimens and swine influenza virus (SIV) tests were common in both respiratory and multisystemic organ system groups. However, organ systems such as gastrointestinal (GI) had a more intuitive pattern where specimen types such as intestine and tests such as electron microscopy and fecal smears were more predominant than in other organ systems. All tests requests (pathology and non-pathology submissions) had up to 6 result fields per requested test and specimen submitted (specimen/test combination). PCR and ELISA test results had a single outcome (positive or negative) per specimen/test combination. The additional result fields included optical density values and s/p ratios for ELISA or CT values for PCR. Tests with more than one possible outcome had multiple outcomes per specimen submitted (multiple specimen/test result combinations). The most common and most complex of these were microbial culture results. "Negative" culture results had outcomes such as "no organisms cultured" or "non-significant organisms". Positive cultures results included non-pathological or opportunistic pathogens such as some serotypes of *E. coli* or species of *Streptococcus.* The additional culture result fields included a score representing the number of colonies per organism identified and a comment field. Antimicrobial sensitivities were not included in the data extract. No further evaluation of test results was conducted for pathology submissions, since their outcomes contributed to the pathology diagnosis.

Table 4: 25 Most common test requests & specimen submitted for Pathology submissions

Tests	Freq.	Per.	Cum. Per	Specimens	Freq.	Per.	Cum. Per
Histology	4565	16.05	16.05	Fixed Tissue (NSP[1])	4540	22.34	22.34
Aerobic Culture	4312	15.16	31.22	Lung	3086	15.18	37.52
PCR PRRS	2520	8.86	40.08	Carcass / Live animal	2509	11.64	49.16
Necropsy - Porcine	2426	8.53	48.61	Pooled lung / tonsil	1360	6.69	55.85
PCR PCV	2318	8.15	56.77	Small Intestine	1295	6.37	62.22
PCR SIV H1N1	1834	6.45	63.22	Spleen	1171	5.76	67.98
PCR Mycoplasma hyopneumoniae	1797	6.32	69.54	Large Intestine	991	4.88	72.86
PCR SIV H3N2	1446	5.09	74.62	Feces (fixed)	857	4.22	77.08
Anaerobic Culture	885	3.11	77.73	Heart	631	3.1	80.18
Electron Microscopy	863	3.04	80.77	Intestine (NSP[1])	620	3.05	83.23
F4 (K88) serotyping	682	2.4	83.17	Culture tube	380	1.87	85.1
PCR Lawsonia spp	559	1.97	85.13	Brain & Spinal cord	359	1.77	86.87
Direct smear	398	1.4	86.53	Kidney	353	1.74	88.61
Coccidia Fecal Smear	359	1.26	87.8	Swab - joint	324	1.59	90.2
PCR Brachyspira pilosicoli	342	1.2	89	Liver	311	1.53	91.73
PCR C perfringens typing	282	0.99	89.99	Fetus	172	0.85	92.58
ELISA Clostridium difficile	273	0.96	90.95	Swab - brain	151	0.74	93.32
PCR Brachyspira hyodysenteriae	231	0.81	91.76	Pooled lung / lymph node	142	0.7	94.02
Gram stain	220	0.77	92.54	Synovial membrane	119	0.59	94.61
FAT TGE	194	0.68	93.22	Nasal swab	118	0.58	95.19
Microminerals	188	0.66	93.88	Fetal stomach contents	116	0.57	95.76
Necropsy – Porcine Fetus	181	0.64	94.52	Tonsil	114	0.56	96.32
PCR PRRS typing	155	0.55	95.06	Skin	111	0.55	96.87
PCR Circovirus typing	142	0.5	95.56	Joint tissue	109	0.54	97.41
PCR Cytomegalovirus (CMV)	142	0.5	96.06	Female Reproductive tissue	105	0.52	97.93

1 Not specified

3.3.3 Pathology Submissions – Diagnoses & Organ systems distributions

All pathology submissions had a primary diagnosis with up to 9 additional diagnoses. 86.4 % of pathology submissions had a primary diagnosis only, one additional diagnosis (primary + secondary) or 2 additional diagnoses (primary + secondary + tertiary) (Figure 4). There were 548 diagnostic codes used 9768 times across the 4726 pathology submissions. Ten diagnostic codes were the most frequently used and accounted for 34.0% of all diagnostic coding.

Systemic PCV2 infections or positive PCR tests for PCV2 (without histological signs) were the two most common codes (11.2%). Five pneumonia codes were included in the most frequent coding: Broncho, broncho-interstitial, interstitial, porcine respiratory and reproductive syndrome (PRRS) virus related and *Mycoplasma hyopneumoniae* related (14.7 percent, collectively). Atrophic enteritis (2.2 %), positive PCR tests for PRRS (2.9%) and "No Specific Diagnosis" (3.0%) were the remaining three frequently used codes.

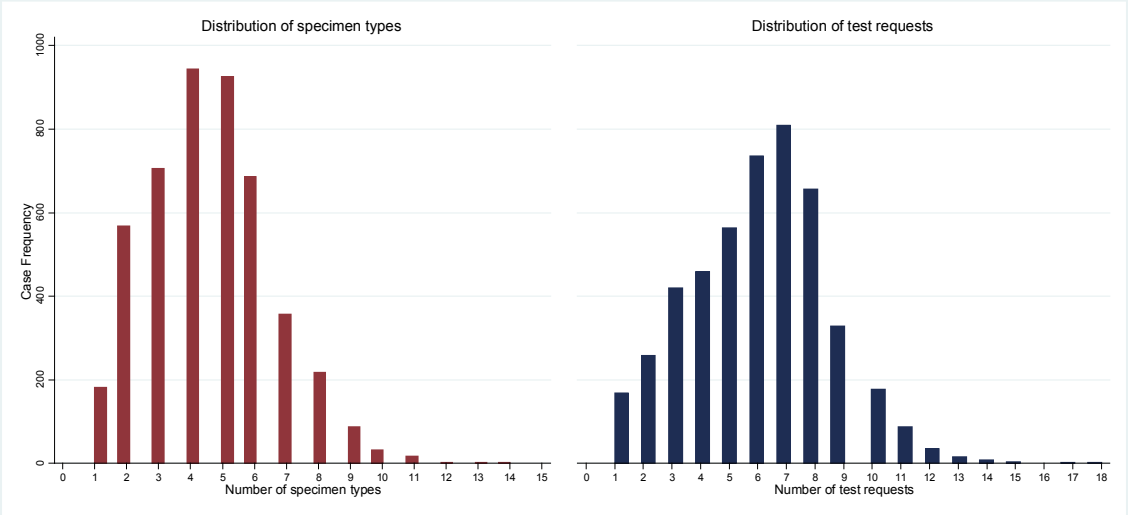


Figure 3: Distributions of Specimen types and Test requests for 4726 Pathology Submissions. The mean number of specimen types per submission was 4.5 with a standard deviation of 2.0 and a range of 1 to 15. The mean number of test requests per submission was 6.0 with a standard deviation of 2.5 and a range of 1 to 18.

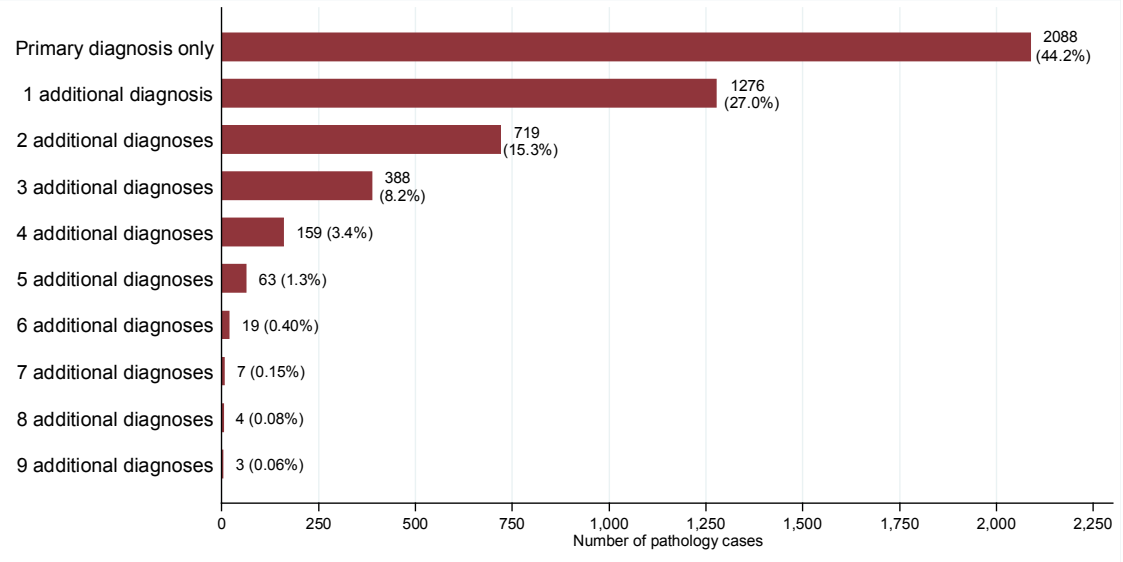


Figure 4: Distribution of 9768 pathology diagnoses across 4726 porcine pathology submissions (cases).

Three organ systems were predominant in pathology submissions across all diagnoses categories: Multisystemic, Lower Digestive and Respiratory (Figure 5). The separate distributions of organ system assignment for primary, secondary and tertiary diagnoses demonstrated the same pattern, with the three organ systems predominating. These findings supported merging Upper and Lower Digestive into a GI system and all of the less common organ systems into an "Other" organ system for further analysis. Note that endocrine system was not assigned to any porcine submissions for any diagnostic category.

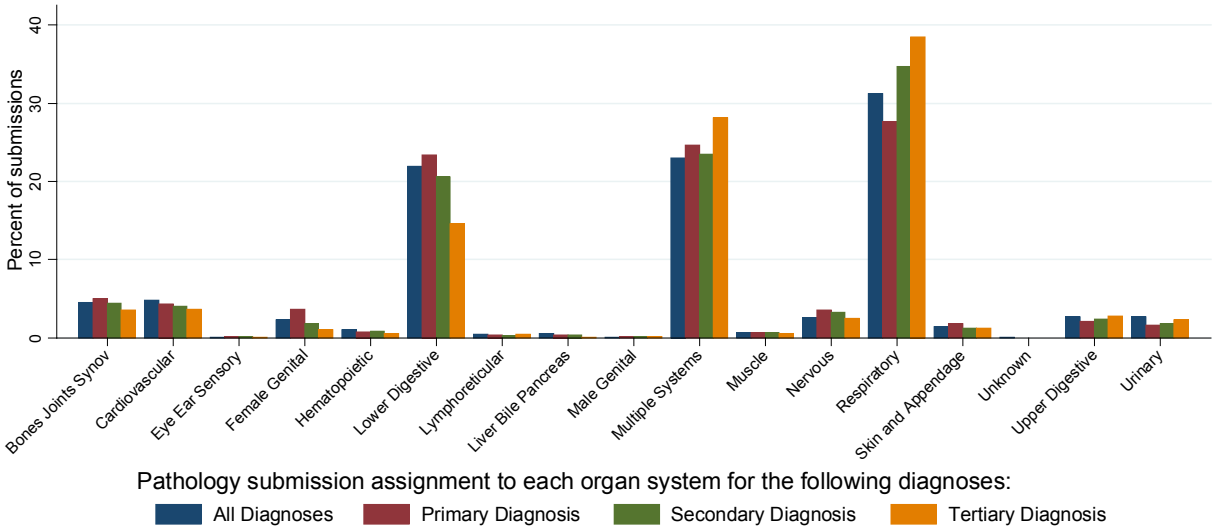


Figure 5: Distribution (by percent) of pathology submissions across all organ systems; for all diagnoses across all categories (9768), for the primary diagnoses category (4726), the Secondary diagnoses category (2636) and the Tertiary Diagnosis category (1362). Note that each diagnosis category is a subset of the category before and each porcine pathology submission has at minimum a primary diagnosis.

To compare with the diagnoses observed across all submissions, Figure 6 shows the 30 most common diagnoses for the grouped organ system classes across the primary diagnoses category. Four, seven and nine distinct diagnostic codes accounted for greater than 50% of the primary diagnosis assigned Multisystemic, Respiratory and GI organ systems, respectively. Ten distinct codes accounted for greater than 40% of the primary diagnosis assigned "Other" organ systems. The ten most common diagnostic codes across the additional diagnoses categories (secondary, tertiary, etc) matched well with the most common codes used in primary diagnoses; PCV2 and "No Specific Diagnosis" codes occurred under the multisystemic category for additional diagnoses. Five of the seven most common primary diagnoses respiratory codes

were also among the ten most common codes across all additional diagnoses. Finally, the most common primary diagnoses GI code, Atrophic enteritis, was also among the ten most common. Due to the combination of many different, less frequently assigned organ systems, the "Other" organ systems did not have a single code or groups of codes that predominated and did not have a code assigned that was represented in the ten most common codes in the additional diagnoses categories. The most common code in "Other", *Streptococcus suis* meningitis, did not exceed 7% of the primary diagnoses assigned.

Additional review was conducted of "No Specific Diagnosis", since similar coding has been used in other surveillance systems to detect emerging disease (Gibbens et al, 2008). "No Specific Diagnosis" was the primary diagnosis in 246 cases and all were categorized as multisystemic. Eighteen had additional diagnoses and of those, nine were PCV2 positive. When compared year over year, "No Specific Diagnosis" in pathology submissions increased from 3.7% and 3.0% in 2003 and 2004 to 6.6%, 6.1% and 5.9% in 2005 to 2007, respectively. By 2008, "No Specific Diagnoses" had decreased to 5.1%.

3.3.4 Pathology Submissions – Clustering of Organ systems categories & Diagnoses variables

Exploratory clustering techniques indicated that additional diagnoses had a significant impact on the grouping of pathology submissions by organ system classification. A three way cross tabulation of the organ system classifications in the primary, secondary and tertiary diagnoses variables supported the likelihood that additional diagnoses may have an impact on the organ system grouping, especially for respiratory and multisystemic categories (Table 5, in yellow): A primary respiratory diagnosis was more likely to have additional secondary and tertiary diagnoses that were either multisystemic or respiratory. In fact the primary respiratory diagnoses often had a secondary respiratory diagnosis (466 cases, 35.7%). A primary multisystemic diagnosis was more likely to have secondary diagnoses of multisystemic (164 cases, 14.1%) or respiratory (250 cases, 19.1%). An additional tertiary respiratory diagnosis was also common (176 cases, 15.1 %) and occurred most frequently with the secondary respiratory or multisystemic diagnoses. A primary GI diagnosis was most likely to have another GI diagnoses as secondary diagnosis (404 cases, 33.6%). A primary "Other" diagnosis was the least likely to have a secondary diagnosis. The most

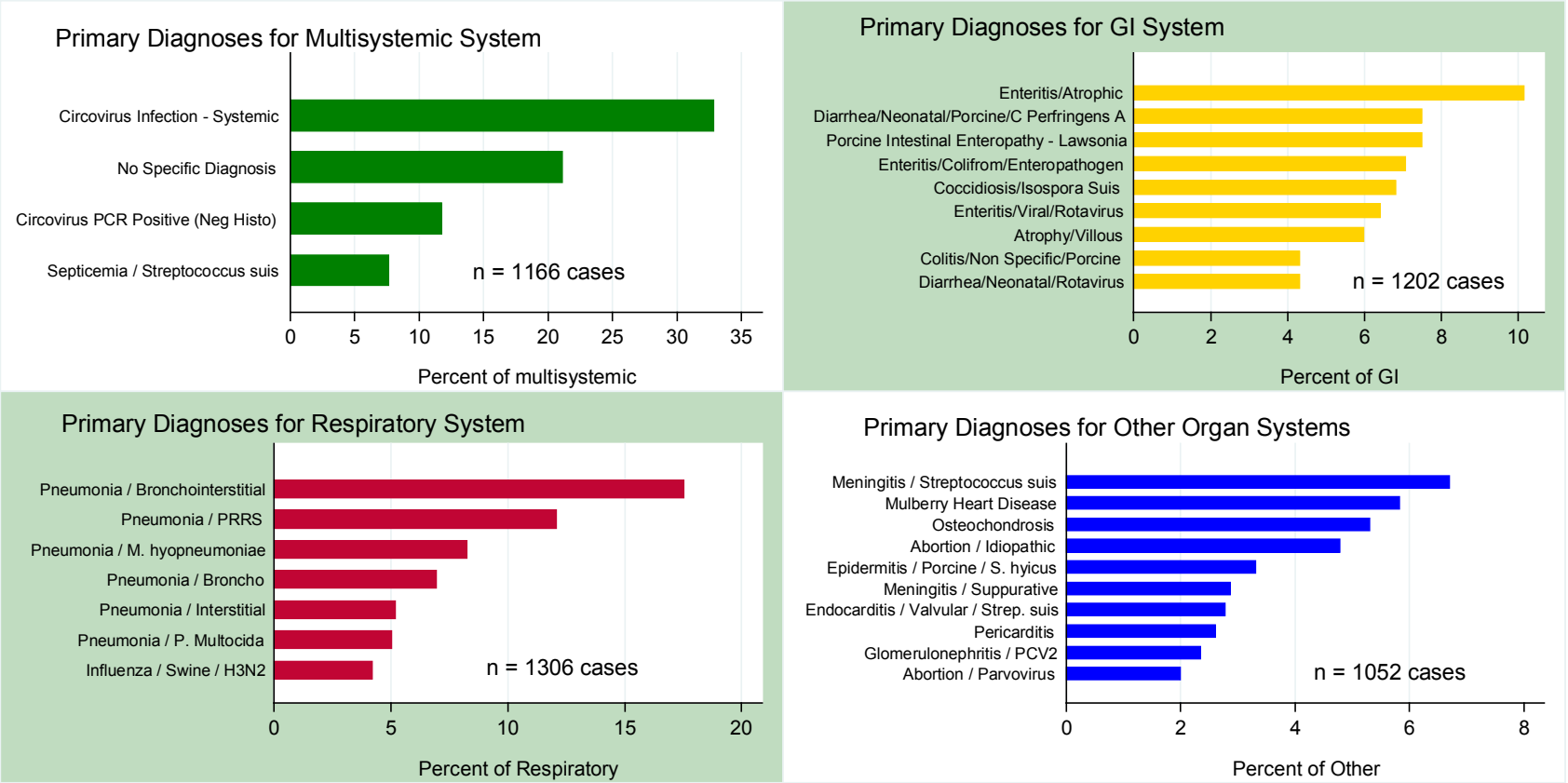


Figure 6: The most common diagnostic codes (in percent) of the primary diagnoses in each of four organ system categories. Diagnostic codes that were used in 4% or more of pathology submissions were included and accounted for 50% or greater of submissions that occurred in each organ system. The exception was “Other” organ systems where diagnostic codes that occurred in 2% or more of cases were included. These accounted for 40% of the total within the “Other” organ systems category.

Table 5: Cross tabulation of organ system categories from secondary and tertiary diagnoses for each primary diagnosis. Percent represents percentage of primary diagnosis

i) Respiratory Primary Diagnosis:

Secondary Diagnoses		Tertiary Diagnoses: GI	MS	Resp.	Other	None	Total
GI	Freq.	19	13	8	6	40	86
	%	1.5	1.0	0.6	0.5	3.1	6.6
MS	Freq.	18	**62**	**53**	18	**99**	**250**
	%	1.4	4.7	4.1	1.4	7.6	19.1
Resp.	Freq.	34	**80**	**122**	32	**198**	**466**
	%	2.6	6.1	9.3	2.5	15.2	35.7
Other	Freq.	15	12	12	20	56	115
	%	1.2	0.9	0.9	1.5	4.3	8.8
None	Freq.	0	0	0	0	**389**	**389**
	%	-----	-----	-----	-----	29.8	29.8
Total	Freq.	86	**167**	**195**	76	782	**1,306**
	%	6.6	12.8	14.9	5.8	59.9	100.0

Pearson chi2(16) = 459.40 Pr = 0.000

ii) Multisystemic Primary Diagnosis:

Secondary Diagnoses		Tertiary Diagnoses: GI	MS	Resp.	Other	None	Total
GI	Freq.	23	1	19	7	45	95
	%	19.7	0.1	1.6	0.6	3.9	8.1
MS	Freq.	16	16	**57**	17	**58**	**164**
	%	1.4	1.4	4.9	1.5	5.0	14.1
Resp.	Freq.	27	39	**72**	36	**76**	**250**
	%	2.3	3.3	6.2	3.1	6.5	21.4
Other	Freq.	9	2	28	14	58	111
	%	0.8	0.2	2.4	1.2	5.0	9.5
None	Freq.	0	0	0	0	**546**	**546**
	%	-----	-----	-----	-----	46.8	46.8
Total	Freq.	75	58	**176**	74	783	**1,166**
	%	6.4	5.0	15.1	6.4	67.2	100.0

Pearson chi2(16) = 597.0310 Pr = 0.000

iii) Gastro-Intestinal Primary Diagnosis

Secondary Diagnoses		Tertiary Diagnoses: GI	MS	Resp.	Other	None	Total
GI	Freq.	**82**	26	24	13	**259**	**404**
	%	6.8	2.2	2.0	1.1	21.5	33.6
MS	Freq.	11	6	13	2	44	76
	%	0.9	0.5	1.1	0.2	3.7	6.3
Resp.	Freq.	7	11	17	3	31	69
	%	0.6	0.9	1.4	0.2	2.6	5.7
Other	Freq.	5	4	6	7	34	56
	%	0.4	0.3	0.5	0.6	2.8	4.7
None	Freq.	0	0	0	0	**597**	**597**
	%	-----	-----	-----	-----	49.7	49.7
Total	Freq.	105	47	60	25	965	**1,202**
	%	8.7	3.9	5.0	2.1	80.3	100.0

Pearson chi2(16) = 393.17 Pr = 0.000

iv) "Other" Primary Diagnosis

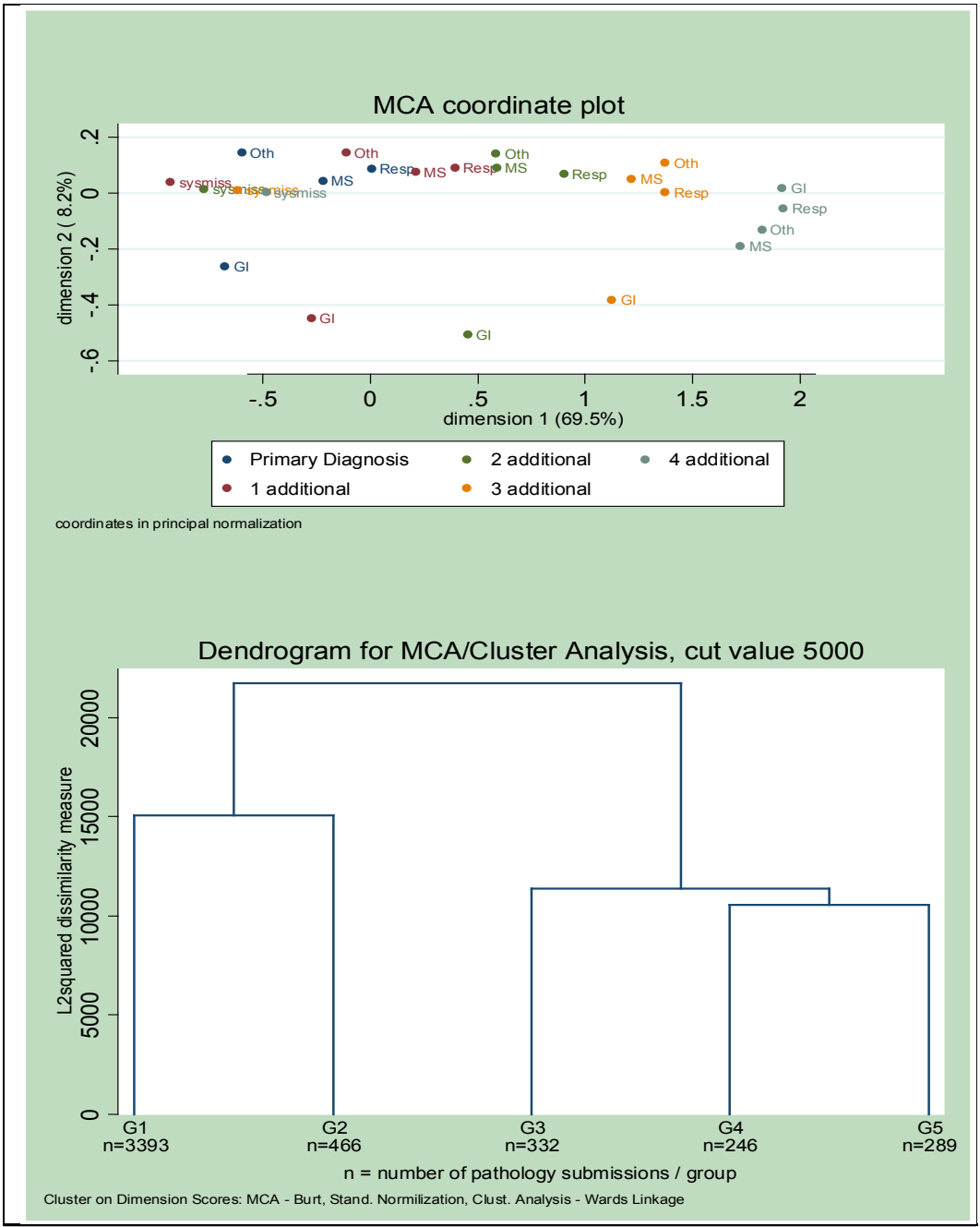
Secondary Diagnoses		Tertiary Diagnoses: GI	MS	Resp.	Other	None	Total
GI	Freq.	6	1	4	10	27	48
	%	0.6	0.1	0.4	1.0	2.6	4.6
MS	Freq.	3	18	11	6	**66**	**104**
	%	0.3	1.7	1.0	0.6	6.3	9.9
Resp.	Freq.	12	17	18	14	**51**	**112**
	%	1.1	1.6	1.7	1.3	4.8	10.6
Other	Freq.	10	21	13	54	**134**	**232**
	%	1.0	2.0	1.2	5.1	12.7	22.1
None	Freq.	0	0	0	0	**556**	**556**
	%	-----	-----	-----	-----	52.9	52.9
Total	Freq.	31	57	46	84	834	**1,052**
	%	3.0	5.4	4.4	8.0	79.3	100.0

Pearson chi2(16) = 402.28 Pr = 0.000

common secondary diagnose with a primary "Other" diagnosis was also "Other" (232 cases, 22.1 %), followed by multisystemic (66 cases, 6.3%) and respiratory (51 cases, 4.8%) diagnoses.

Tertiary diagnoses were less likely to occur with either primary GI or "Other" diagnoses categories, than with primary respiratory or multisystemic. When they did occur, they tended to be in the same category as the primary diagnoses. While the 3 way tabulation did indicate considerable secondary and tertiary diagnoses, the evaluation also indicated that the singular most frequent occurrence for all four primary diagnoses was no additional diagnoses (None). The values ranged from 389 cases (29.8%) for respiratory to 556 cases (52.9%) for "Other".

The results from the MCA and cluster analysis indicate the impact additional diagnoses had in organ system clustering depended on the number of additional diagnoses categories included and the weight given to additional diagnoses "missing" for each case submission. The inclusion of 4 additional diagnoses and "missing" additional diagnoses suggest that submissions cluster on whether there is primary diagnosis only or additional diagnoses, regardless of what organ system was assigned (Figure 7). The MCA explained greater than 85% of the total inertia in 6 dimensions. The first dimension inertia contributed the most at 69.5%. Clustering on the first dimension axis in the MCA plot appeared to be primarily based on the presence or absence of any additional diagnoses; missing values cluster nearest primary diagnoses and are farthest from the third and fourth additional diagnoses. The missing values (sysmiss) are also closest (on the first dimension axis) to "Other" and GI, which have the highest percentage of primary diagnosis only. Clustering of primary diagnosis only (regardless of organ system category) versus additional diagnoses is further demonstrated in cluster group 1 (Cluster Analysis, squared dissimilarity measure cut point at 5000), which has a very distinct branch on the dendrogarm and includes most of the primary diagnosis category, across all assigned organ systems (15000 squared dissimilarity measure). Note that the higher the squared dissimilarity measure of the branches, the greater likelihood that the clusters are truly distinct. These findings correspond to the high percentage of primary diagnosis only in the cross tabulations (Table 5). Clustering effect of actual additional diagnoses appears to have much less impact. The second dimension inertias contributed only 8.2% and the clustering of additional diagnoses appears to occur primarily on this axis. However, some effect can be noted as respiratory and multisystemic and "Other" organ system assignments for 1 additional and 2 additional diagnoses cluster near primary respiratory and primary multisystemic organ systems. Additional GI diagnoses are also seen to occur at similar 2 dimension axis values as primary GI diagnoses. To limit the impact of too many "missing" diagnoses, further MCA and cluster



Organ system clusters[1], Primary Diagnosis

	Cluster					
Diagnosis	**G1**	**G2**	**G3**	**G4**	**G5**	**Total**
Respiratory (Resp)	931	60	161	70	84	1306
Gastro Intestinal (GI)	738	309	29	24	102	1202
Multi Systemic (MS)	856	65	99	72	74	1166
Other (Oth)	868	32	43	80	29	1052
Total	3393	466	332	246	289	

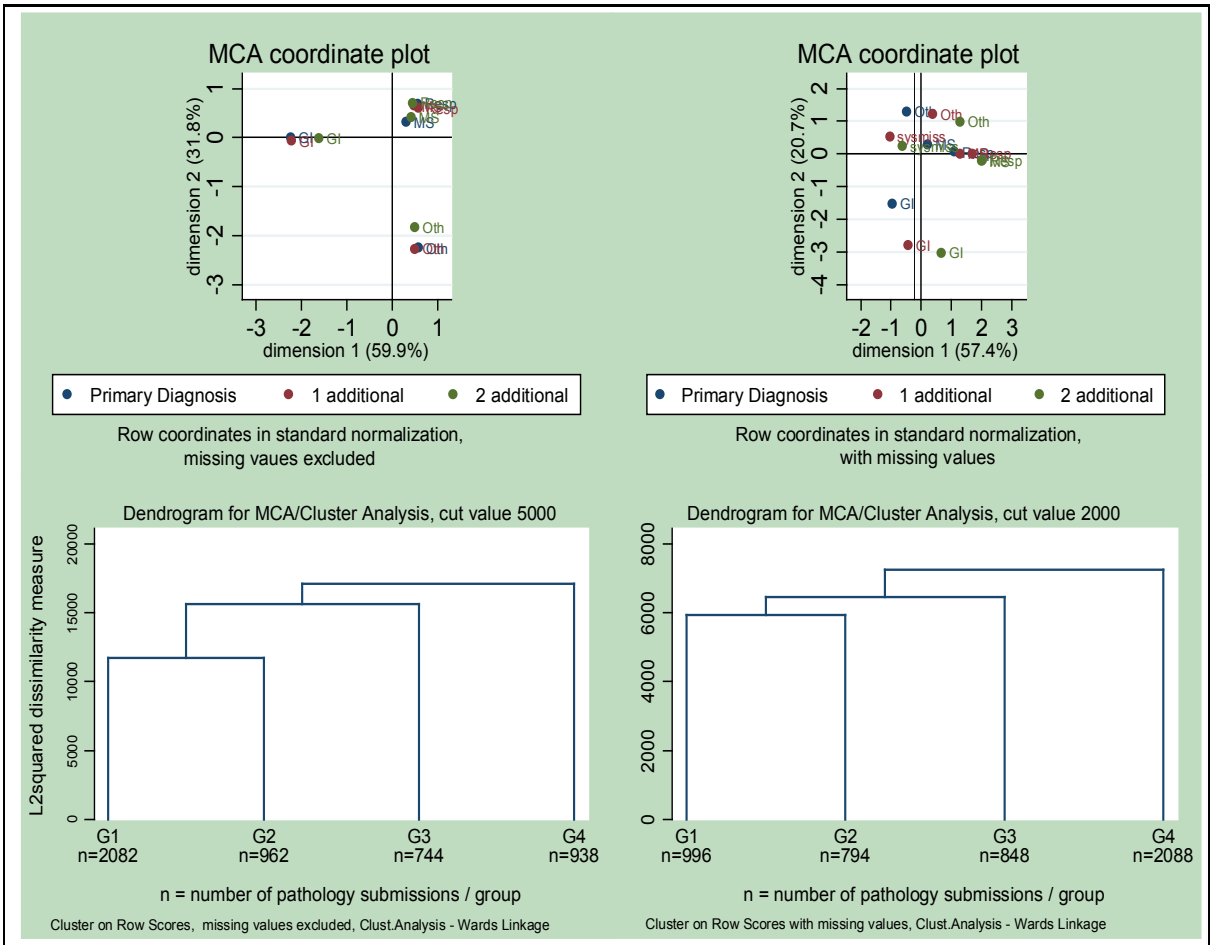
1. Cluster on dimensions scores; MCA – Burt matrix, standard normalization. Cluster analysis – Ward's Linkage

Figure 7: MCA/Cluster analysis using dimension coordinates, 4 additional diagnoses with missing values included (sysmiss). 3 dimensions included (2 displayed)

analysis was limited to the primary plus 2 additional diagnoses or conducted by excluding missing values or both (most pathology submissions were concluded with 2 additional diagnoses or less, see figure 5). Cluster analysis on dimension coordinates appeared to place too much emphasis on missing values in the additional diagnoses variables even if the additional diagnoses were limited to 2. The results (not shown) were similar to those from 4 additional diagnoses displayed in Figure 7. Subsequent cluster analyses were limited to row scores.

The results from MCA and cluster analysis using row coordinates from up to 2 additional diagnoses, excluding and including missing values are presented in Figure 8. With missing values for additional diagnoses included, 85% and 86% of the total inertia was explained within third and fourth dimension respectively. The second dimension values contributed over 20% to the total inertia, suggesting a greater contribution from the real additional diagnoses. The MCA plot suggested "Other" and GI diagnoses were more distinct than in the previous analysis. Respiratory and multisystemic diagnoses appear to have emerged as one cluster. The missing values (sysmiss) seem to still have an impact as the primary diagnosis variables tend to align between these values and existing additional diagnoses. The cluster analysis (squared dissimilarity measure cut point of 2000) produced 4 distinct groups with significant branches occurring above 5000. Even with the relatively low cut point, missing values had a significant impact, with the largest group (group 4) having missing values in all the additional diagnoses variables. The other groups did each tend to have a predominant organ system but also had a significant number of submissions with other organ systems present. With missing values for additional diagnoses excluded, greater than 94% of the total inertia was explained within 3 dimensions. The second dimension inertias contributed more in this analysis (31.8%) than in the previous analysis. The greater emphasis on existing organ systems assignments in the additional diagnoses variables appears to have had a significant impact on the MCA. The MCA plot shows three distinct groups where multisystemic and respiratory primary diagnoses cluster together as one group while GI and "Other" remains distinct. The plot is comparable to the high number of respiratory and multisystemic additional diagnoses for both respiratory and multisystemic primary diagnoses seen in the cross tabulation. Also similar to the cross tabulation was the distinct groups of primary GI plus additional GI and primary "Other" plus additional "Other" diagnoses. The cluster analysis (squared dissimilarity measure cut point of

5000) produced four distinct groups, with significant branches occurring above 10000. Group 1 was predominantly a respiratory group as it included all primary respiratory diagnoses. It also included the majority of the additional diagnoses that were either respiratory or multisystemic. GI and Other primary diagnoses in group 1 were unique in that at least one of the additional diagnoses was not the same organ system as the primary diagnoses. The multisystemic primary diagnoses in group 1 were not as well defined. Although it had additional diagnoses, some submissions were all multisystemic. Adjusting the dissimilarity measure cut point to 3000 split group 1 into two new groups; the majority of the primary respiratory diagnoses and a small number of primary multisystemic diagnoses separated into a new group. The remaining group was made up of primary diagnoses that had at least one additional diagnosis that was a different organ system than the primary diagnoses. Excluding the missing analysis allowed the existing additional diagnoses to have a greater impact on the overall inertia and subsequent clustering. Lowering the cut point allowed the generation of a separate respiratory group suggesting that submissions with primary respiratory diagnoses are affected the most by additional diagnoses. The results from the MCA/cluster analysis with row scores and excluding missing values results provide two possible sets of outcomes for further syndromic classification of pathology submissions, depending on the dissimilarity cut point used.



Organ System Clusters by Primary Diagnosis

	Cluster, missing excluded					Cluster, missing included				
Diagnosis	**G1**	**G2**	**G3**	**G4**	**Total**	**G1**	**G2**	**G3**	**G4**	**Total**
Resp	1,306	0	0	0	1,306	614	132	171	389	1,306
GI	264	0	0	938	1,202	0	531	74	597	1,202
MS	204	962	0	0	1,166	318	131	171	546	1,166
Oth	308	0	744	0	1,052	64	0	432	556	1,052
Total	**2,082**	**962**	**744**	**938**	4,726	**996**	**794**	**848**	**2,088**	4,726

Figure 8: Side by side comparison of MCA and cluster analysis of row coordinates from up to 2 additional diagnoses (Primary, Secondary and Tertiary diagnoses variables) using 3 dimensions (2 displayed). Missing values excluded (left) and missing values included (Right).

3.3.5 Non-Pathology Submissions

Porcine non-pathology submissions included 66 test codes and 85 specimen codes. The most common 25 test requests accounted for greater than 96% of all test requests and the most common 25 specimens submitted accounted for greater than 95% of all specimens submitted (Table 6). The median number of specimen types submitted was 1, the 25th and 75th

percentiles were also 1 and the range was 1 to 15. As evident with the limited distribution of specimen types (Figure 9), the majority of non-pathology submissions involved a single specimen type. The predominant specimen type was sera, which accounted for over 52% of the specimen types and corresponded to the specimen of choice for 6 of the top 7 test requests in table 6. The average and median numbers of sera samples per non-pathology submission was 32 and 20 respectively. The non-pathology test requests were also limited, with a median number of test requests per submission of 2, with a 25th percentile of 1, a 75th percentile of 3 and a range of 1 to 13.

The predominant test requests were more specific and focused on significant endemic diseases, especially for respiratory/reproductive diseases such as PRRS and respiratory diseases such as *Mycoplasma hyopneumonia* and Swine Influenza Virus. These tests make up 10 of the top 13 test request for non-pathology submissions. Tests for Transmissible Gastroenteritis (TGE) and Porcine Circovirus (PCV) were also frequently recorded. Besides test request for separate diseases, the frequency of different types of tests for a specific disease was also noted. Two testing methods for the same disease maybe requested for the same submission (e.g. PCR and ELISA testing for PRRS). As noted for pathology submissions, test results had one or multiple outcomes depending on the test requested.

Unlike pathology submissions, there were no additional outcomes (pathology diagnoses) to determine if a non-pathology submission was positive or negative for a specific disease. For example, many culture results were positive for ubiquitous or opportunistic organisms that may or may not be the cause of disease. Tests such as ELISA may have been positive in response to vaccination instead of disease. Additional submission information, such as clinical history or reason for submission, was not available to further determine if a test result was significant for a non-pathology submission. Test sensitivities and specificities for tests such as ELISA and PCR, were available, as well as the numbers of specimens submitted. In combination with estimated disease prevalence for certain diseases in the Manitoba swine population, these values were used to extrapolate whether a non-pathology submission involving those tests was positive. The results are not presented in this study.

Table 6: 25 Most common test requests & specimen submitted for Non-pathology submissions

Tests	Freq.	Per.	Cum. Per	Specimens	Freq.	Per.	Cum. Per
PCR RT PRRS	7866	21.43	21.43	Serum	10599	52.54	52.54
ELISA PRRS[1]	7659	20.86	42.29	Swab (PRRS specific)	2300	11.4	63.95
ELISA M. Hyopneumoniae	5328	14.51	56.8	Lung	1184	5.87	69.81
ELISA TGE / Respiratory coronavirus	2339	6.37	63.17	Semen (Fresh)	1166	5.78	75.59
Aerobic Culture	2322	6.33	69.5	Semen (Extended)	520	2.58	78.17
ELISA Porcine SIV H1N1	1854	5.05	74.55	Fixed Tissue (NSP[2])	442	2.19	80.36
ELISA Porcine SIV H3N2	1149	3.13	77.68	Environmental sample	342	1.7	82.06
PCR PRRS	911	2.48	80.16	Nasal Swab	289	1.43	83.49
IFA PRRS	909	2.48	82.64	Feces (fresh)	287	1.42	84.91
PCR SIV H1N1	801	2.18	84.82	Carcass	264	1.31	86.22
PCR PCV	793	2.16	86.98	Spleen	246	1.22	87.44
PCR M. hyopneumoniae	666	1.81	88.79	Heart	199	0.99	88.43
PCR SIV H3N2	648	1.77	90.56	Small Intestine	196	0.97	89.4
Histology	438	1.19	91.75	Pooled lung / tonsil	177	0.88	90.28
Necropsy - porcine	268	0.73	92.48	Large Intestine	127	0.63	90.91
Semen evaluation	223	0.61	93.09	Feces (fixed)	125	0.62	91.53
Other Reference Laboratory Referrals[3]	196	0.53	93.62	Fecal Swab	123	0.61	92.14
PCR PRRS typing	188	0.51	94.14	Bacterial culturette Swab	106	0.53	92.66
PCR Lawsonia	188	0.51	94.65	Intestine (NSP[2])	106	0.53	93.19
F4 (K88) serotyping	170	0.46	95.11	Liver	103	0.51	93.7
Anaerobic Culture	160	0.44	95.55	Culture tube	95	0.47	94.17
Fecal flotation	131	0.36	95.9	Urine	79	0.39	94.56
Electron Microscopy	125	0.34	96.24	Swab - joint	74	0.37	94.93
PCR Brachyspira pilosicoli	95	0.26	96.5	Kidney	68	0.34	95.27
General Chemistry	89	0.24	96.74	Brain & Spinal cord	55	0.27	95.54

1 Includes both Dako and Idexx PRRS ELISA test kits
2 Not specified
3 VDS includes test codes for common diagnostic tests sent not conducted at VDS, such as Electron Microscopy. Less common referrals to another laboratory are placed in an “Other” category

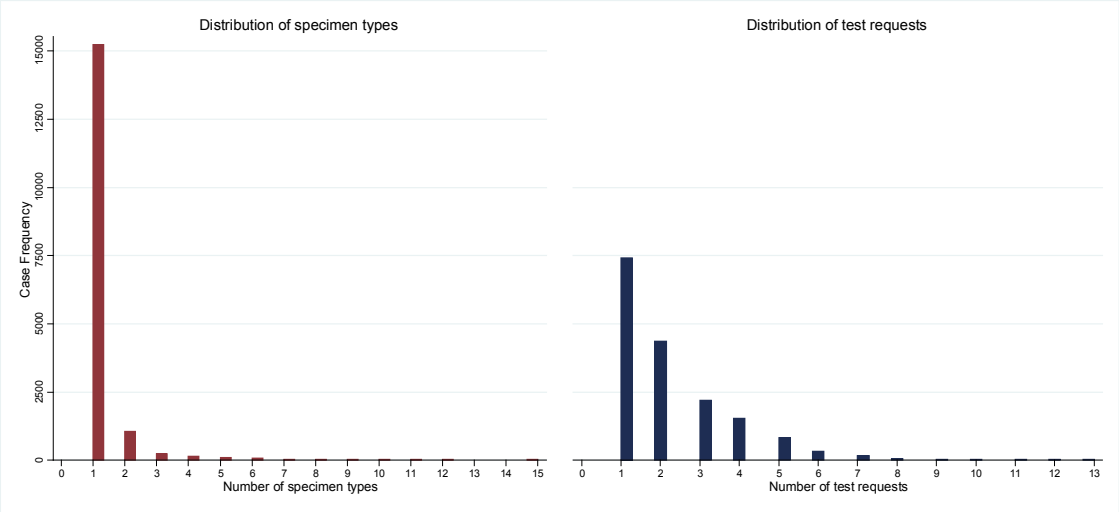


Figure 9: Distributions of Specimen types and Test requests for 16939 Non-pathology Submissions. The median number of specimen types per submission was 1, with the 25th and 75th percentiles also equal to 1 and a range of 1 to 15. The median number of test requests per submission was 2 with a 25th percentile of 1, a 75th percentile of 3 and a range of 1 to 13.

3.4. Discussion

Evaluation and analysis of a laboratory data source conducted in this chapter follows the approaches taken by others in developing, implementing and validating syndromic surveillance methods in animal health (Dorea et al, 2011; Dupuy et al, 2013a). The data source and focus on a regional swine population is an example of how the broad range of activities in animal health, especially in food animal production, plus the increase in electronic data capture have provided a variety of sources to draw data from for syndromic surveillance. However, it is also an example of how the wide range in species and associated diseases, the semi-structured nature of the data, the lack of a generally accepted standardized disease nomenclature and the multi level clustering of food animal populations are complexities that have led to a variety of approaches to syndromic surveillance in animal health (Dorea et al, 2011; Dupuy et al, 2013a).

A syndromic surveillance system should be sensitive, efficient and timely in order to be effective; it should be able to consistently identify real disease clusters early enough for an effective response (Guasticchi et al, 2009). Two key assumptions that underlie a syndromic surveillance system are: a) significant disease information from the population under surveillance is in the data available (Dorea et al, 2011); the data are either representative enough of the population or at minimum, representative for the at risk groups within the

population; b) Real disease clusters within the population under surveillance are detectable by the syndromes established within the system. Syndrome classification and validation are important methods that assist in supporting the assumptions and developing a syndromic surveillance system (Chapman et al, 2005b; Guasticchi et al, 2009; Kashiouris et al, 2013; Leal and Laupland, 2008). Appropriate classification of syndromes identify significant disease clusters and are established (or limited) by the data source and type (Buehler et al, 2004). The comparison of syndromes to representative groups of diagnostic outcomes establishes the sensitivity and specificity of the syndromes (Chapman et al, 2005b; Guasticchi et al, 2009; Van Metre et al, 2009). The evaluation of porcine submissions to VDS has provided information of the availability and limitations of the test requests and specimen types for syndrome classification. The analysis has also provided the basis for grouping pathology diagnoses into usable clusters for syndrome validation.

The review of yearly and seasonal trends observed in porcine submissions to VDS indicated variations that were not unexpected and may have occurred for several possible reasons. Porcine circovirus associated disease (PCVAD) caused by a variant strain of PCV2 significantly impacted the health of the Canadian swine herd from 2004 to 2008 (Carman et al, 2008; Gagnon et al, 2007; Poljak et al, 2010). In Manitoba, the number of farms reporting PCVAD increased from 13 in 2005 to 172 in 2006 and 159 in 2007. In response to the outbreak, a government sponsored Porcine Circovirus inoculation program was available to Canadian pork producers in 2007. The program required diagnostic testing and veterinary confirmation of disease for the producer application. PCVAD and diagnostic testing for the inoculation program were likely contributors to the increase in non-pathology submissions from 2004 to 2007. The introduction of PCVAD vaccine and the vaccine uptake supported by the inoculation program were likely contributors to the decrease in submissions that followed. A follow up review of monthly submission data from VDS supported this conclusion; PCVAD testing peaked at over 30 submissions per month in the fall of 2005 with a secondary peak of over 20 submissions per month from January to April 2007, corresponding to the initial introduction of the vaccine program (and subsequent test requirements). By June 2007 PCVAD testing had declined to less than 10 submissions per month and by March 2008 the average fell to less than 5 per month. Additional contributors to the overall decrease in submissions were three key economic factors that negatively impacted swine production in Manitoba in 2008 and 2009: a) A high Canadian

dollar, which has been demonstrated to significantly alter porcine submissions to another regional animal health laboratory (O'Sullivan et al, 2012). b) Full implementation of Country of Origin Labeling (COOL) legislation in the US in 2008 (Thevenaz 2011). c) elevation of corn prices in the main North American pork feeding sectors (Hofstrand 2009). Finally an environmental review of the Manitoba swine industry in 2007 led to tighter regulatory control and an overall decrease in production in subsequent years (Anon 2007). Overall, the VDS submission rate trends suggest that a significant disease event (PCVAD) may be detectable within the data. However, factors outside of animal health, such economic viability, environmental sustainability and regulatory control may impact veterinary involvement with swine health and subsequent submission rates to a regional laboratory (O'Sullivan et al, 2012). The impact appears greater for non-pathology submissions; non-pathology submissions include non-diagnostic testing (testing for confirmatory health status, sale/export, vaccination response) which is likely impacted more by external factors that affect swine farm profitability. Awareness and consideration must be given to these external factors when considering submission trends and the use of non-pathology submissions for syndromic surveillance.

The results of the evaluation indicate that test requests and specimen types from pathology submissions may form the basis of pre-diagnostic groups for syndrome development. The distribution of test requests and specimen types indicated there were several specific tests (e.g. PCV, PRRS, and Electron Microscopy) and specimens (Lung, Intestine) that occurred more frequently in some pathology diagnoses than in others. As well pathology submissions on average appeared to have adequate numbers of both specimens and tests to draw classifications from. Both of these findings support the use of test requests and specimen types for syndrome classification. However, it is recognized that many of the test requests and specimen types were very common and non-specific, such as carcass, fixed tissue, histology and necropsy. As a result, classification methods that utilize combinations and patterns of specimen types and test requests, plus allow additional elements such as number of specimens submitted are likely the appropriate approach to syndrome development.

Utilizing organ systems to cluster pathology or clinical diagnoses into outcome groups for syndrome validation or comparison is consistent with other syndromic surveillance approaches using animal health laboratory data (Amezcua et al, 2013; Dorea et al, 2013; Gibbens et al, 2008; Hyder et al, 2011). For example, Amezcua used the most common reported organ

systems, Respiratory, Digestive and Reproductive to compare a practice based swine syndromic surveillance system to laboratory submissions (Amezcua et al, 2013). The analysis of pathology diagnoses has identified three possible options for outcome clusters based on organ system assignment: Four groups based on primary diagnoses only, four groups (G1-G4) based on cluster analysis branched at a squared dissimilarity measure of 10000 (Ward's linkage) and five groups (G1-G5) branched at a squared dissimilarity of 5000 (Ward's linkage). Diagnostic outcomes based on primary diagnoses only do have merit, since the primary diagnoses were considered the most significant. However, this method ignores patterns in the pathology data where a primary diagnoses may be consistently linked to a secondary diagnosis. For example, primary respiratory diagnoses were often followed by a secondary multisystemic diagnoses, which on closer inspection, was often linked to PCVAD. The four group cluster and the five group cluster offer the better diagnostic outcome options as both clusters recognize that the GI and "Other" organ system classifications occur primarily as single diagnoses or with secondary diagnoses of the same organ system. Both clusters also identify a considerable number of multisystemic as a separate group. A separate multisystemic organ system group that includes submissions with a primary multisystemic only or primary multisystemic plus secondary multisystemic may be important for emerging disease detection; it would include all "No Specific Diagnoses" and a considerable number of the PCVAD diagnoses. However, the inclusion of a fifth group that is primarily respiratory may be an artifact of the cluster method for several reasons; the branching occurred at considerably lower values than the other groups, it is not supported by the MCA, (which appeared to suggest three groups) and, as evident from the 3 way cross tabulation, the primary respiratory diagnoses did tend to occur with more additional diagnoses than the other primary organ system diagnoses. It appears that the most appropriate diagnoses outcome group from the MCA and hierarchical analysis was four clusters (G1-G4) with "missing" diagnoses excluded. On closer inspection, cases within the first cluster (G1) were consistent with cases that had a primary respiratory diagnosis alone or with additional diagnoses, as well as with cases that were primarily multisystemic, GI, or "Other" but had additional diagnoses that were not equivalent to those primary diagnoses. Cases within the clusters G2 to G4 were consistent with cases that had both a primary and additional diagnoses of multisystemic, "Other" and GI respectively.

Methods that excluded “missing” diagnoses from MCA and hierarchical clustering provided a more accurate representation of diagnostic outcomes. The impact of “missing” diagnoses was due to the hierarchical nature of the diagnoses variables; a pathology submission typically only needed up to 2 additional diagnoses (86.4% of all submissions) and the inclusion of each additional diagnosis increased the number of “missing” diagnoses significantly. The cluster analysis with “missing” diagnoses included does appear to support a case definition of primary diagnoses only, since single large clusters occurred that included all organ systems within them. This is likely because the “missing” diagnoses dominated the inertia in the first dimension in the MCA, leading the cluster analysis to appear to give equal or greater weight to a branching based on “missing” versus non missing diagnoses instead of what the diagnoses actually were. Applying equal or greater weight to “missing” diagnoses is not representative of how the diagnoses are reported in the data set. It was noted that when missing values were included, cluster analysis on row scores appeared more realistic than on dimensions scores. Row scores put greater weight on observations and less on variables so additional diagnoses variables are not over weighted.

Creating an “Other” category for organ systems that are assigned at a lower frequency poses problems for identification of significant reportable or emerging diseases using laboratory based syndromic surveillance. For diseases such as pseudorabies and classical swine fever specimen types and test requests may indicate neurologic and skin pathology. In theory a laboratory based syndromic surveillance system may not be sensitive enough to detect these diseases if the submissions are mixed with a large diverse organ system case definition. However, many of these diseases also exhibit respiratory and multisystemic components. Perhaps more importantly, reportable diseases tend to spread very rapidly and have significant clinical signs in naive populations. If multiple types of surveillance are in place, such as clinic based and abattoir base surveillance, then there is a greater likelihood disease such as these would be detected by other means (Amezcua et al, 2013; Dupuy et al, 2013b).

Specific diagnostic coding of disease conditions (Figure 6) in the VDS’ LIMS data provide an alternative method of clustering diagnostic outcomes not explored in this study. Using the larger number of more specific disease codes would provide two opportunities for syndromic surveillance. First, the analysis of clusters within diagnostic codes may have provided more refined pathology outcomes to validate syndrome classification from submitted test requests

and specimen types. Similar techniques have been used to group diagnostic codes for validation in public health syndromic surveillance systems (Betancourt et al, 2007; Chapman et al, 2005b; Guasticchi et al, 2009; Ivanov et al, 2002). Second, the diagnostic codes may also provide a means of evaluating and grouping pathology outcomes into effective indicators or syndromes for early warning surveillance, instead of using pre-diagnostic syndromes. This approach is similar to those proposed in studies that have evaluated full and partial carcass condemnation data from abattoirs for the purpose of grouping condemnation outcomes into syndromes (Alton et al, 2010; Alton et al, 2012; Dupuy et al, 2013b). In one study, condemnation outcomes including coded reasons for condemnation, condemned portions, slaughter date, animal factors (age, production type, sex) and abattoir factors (location, days operating, mean number slaughtered per day) were clustered into syndromes using MFA, including MCA and hierarchical clustering (Dupuy et al, 2013b). Even more importantly, the approach has been applied in the United Kingdoms' Veterinary Diagnostic Analysis System where diagnostic outcomes from animal health laboratory submissions are grouped for surveillance purposes. The UK system uses established disease codes to conduct surveillance on the incidence of known diseases and conditions as well as "Diagnosis Not Reached" codes for new and emerging diseases (Gibbens et al, 2008; Hyder et al, 2011; Kosmider et al, 2011). Following the UK example, a combination of disease and organ system codes in the VDS LIMS may also provide more refined method of evaluating "No Specific Diagnoses". This is an important consideration since "No Specific Diagnosis" may have been an early indicator of PCVAD in Manitoba; the yearly trend data suggested that during the initial emergence of PCVAD, the "No Specific Diagnosis" code was reported at almost twice the rate; 6.3% for 2005-2007 compared to 3.4% for 2003-2004. While the diagnostic coding within the VDS LIMS is worth exploring using either of the two approaches described above, a key limitation compared to other systems must be considered. VDS disease codes do not follow any specific standardized disease nomenclature mentioned earlier (e.g. LOINC) and the rules for the inclusion or exclusion of codes are not externally reviewed. Diagnostic codes may be added or removed based on a less formalized internal review process. This is consistent with one inventory of veterinary syndromic surveillance systems, where the use of internal coding systems was the norm if coding systems were used at all (Dupuy et al, 2013a). However, it is generally accepted that formal, standardized methods of disease coding in public health and in

the UK Veterinary Diagnostic Analysis System provide a more stable disease nomenclature for surveillance purposes (Dorea et al, 2011; Dupuy et al, 2013a).

The use of non-pathology submissions for early indications of emerging or re-emerging disease in swine herds will be more complex because additional factors such as reason for submissions and specific diagnoses are not available. Surveillance using laboratory submission data without specific diagnoses has relied on reason for submission (Gibbens et al, 2008), specific counts of test results (Shaffer et al, 2008), text mining of case descriptions and laboratory comments, and/or identification of pathogen specific tests (Dorea et al, 2013). Coded reasons for submissions and submitter comment fields were not available in the data extract for use. However, if submitting veterinarians provided a detailed case history, then text mining of additional fields within the LIMS could be used to identify key words and mapped to rule-based syndromes using a supervised classification algorithm (Chapman et al, 2005a; Dorea et al, 2013). Using counts of positive test results or isolates of specific microorganisms to establish case outcomes are more difficult to interpret when the health event is measured at the herd or group level. To account for herd level effects, the number of samples submitted per event, estimates of herd level prevalence and the sensitivity/specificity of the tests involved need to be included in the case definition. Additionally, submission bias is more likely to occur from swine herd level testing because the reason(s) for non-pathology submissions are frequently non-diagnostic. For example, PRRS testing is frequently used to confirm the stability of the virus in a positive herd without clinical signs, to estimate the effect of a vaccination strategy or to confirm negative herd status for sale or export. In these cases, the selection bias arises for syndromic surveillance because the samples are submitted from healthy animal groups, not diseased groups. Without identifying the reason for submission, submission bias would significantly affect both counts of tests results and identification of pathogen specific tests. It may be possible to modify the syndrome selection criteria to identify submission patterns that are typically non-diagnostic and decrease selection bias. For example, a case submission with 50-100 sera samples for limited testing is more likely conducted for sale or export where individual animal status is as important as herd status. As well, there are specific sampling protocols that are meant for herd status determination and less for diagnostics (Holtkamp et al, 2010). Identifying non-pathology submissions with these patterns as “non-diagnostic” may benefit a syndromic classification scheme in the absence of reason for submissions.

Unfortunately, methods of evaluating diagnostic outcomes and pre-diagnostic data for syndrome classification and validation cannot be easily transferred to other animal health laboratory or veterinary data bases. The data source in this study is another example of a key reason why; the lack of standardization in disease nomenclature across all animal health data bases. The consistent use of standardized disease nomenclature in public health databases has improved the ability to establish syndromic systems in similar data sources and has made the interoperability of multiple syndromic systems possible (Heffernan et al, 2004; Lombardo et al, 2004; Wagner et al, 2004). In Canada, the establishment of broad based animal health networks on a public health platform and the recommendation of a minimum data set for collation and analysis of diagnostic laboratory data are seen as steps to address the lack of standardization across many animal health data sources (Kloeze et al, 2010; Kloeze et al, 2012). The objectives within these steps also recognize that even when data sources and data coding may be similar, different methods may be necessary due to differences in data structure and availability, differences in animal populations between regions, or differences between species of animal under surveillance (Kloeze et al, 2012). For example, Dorea evaluated an animal health laboratory data source in Ontario, Canada for syndrome classification of bovine submissions. The evaluation led to the exploration of rule-based and automated machine learning methods applied to 75% of all bovine test requests instead of pathology submissions only (Dorea et al, 2013). However, it was recognized that further research was necessary to establish ongoing validation of the classification methods. By comparison, the evaluation conducted in this study led to a focus on pathology submissions as there was support in the data and the literature for an assumption these were more representative of clinical cases in swine, including atypical ones and less influenced by external non-disease factors (Amezcua et al, 2013; Dorea et al, 2011; Gibbens et al, 2008; O'Sullivan et al, 2012). The evaluation also recognized that clustering pathology diagnoses into defined outcomes provided a simplified practical basis for syndrome validation and updating of syndrome classification. However, it is recognized that further research is needed into syndromic methods applied to non-pathology submissions as they represent the bulk of swine submissions and the assumption regarding clinical cases will not always apply.

The overview, evaluation and analysis of the VDS laboratory data has described the information available for the development and implementation of syndromic surveillance methods. It is

important to note that the chapter did not specifically evaluate the timeliness of syndromic methods over conventional reporting for either pathology or non-pathology submissions. Further research into the timeliness of the data as part of time series analyses and aberration detection methods would be considered necessary. However, the chapter purpose has been specifically addressed in the following ways:

- From the yearly and seasonal trends, it is possible that changes in the health of the regional swine population may be detected from the data source over time, specifically the incursion of PCVAD and subsequent animal health program response. However, the trends also demonstrated potential impacts of three economic and one regulatory factor.
- From the description of laboratory submission data, it was noted that requirements to meet a minimum data set for surveillance were not readily available (Kloeze et al, 2012). In particular, cases were not classified by the submitter into body system classifications for syndromic surveillance. However, consistent with the evaluation of other laboratory data sources, the test requests and specimen types were instead the most structured and useful components available to form syndrome groupings.
- From the evaluation test requests and specimen types between pathology and non-pathology submissions, the greater numbers and types of specimens and test requests in pathology submissions supported the expectation of differences in reasons for submissions and the greater likelihood of submission bias in non-pathology submissions. The differences also support the assumption that pathology submissions are more representative of typical (and atypical) clinical swine cases. From the assessment of laboratory results and pathology diagnoses, grouping laboratory results from non-pathology submissions into diagnostic outcomes for syndrome validation would involve more complex exploratory and analytical methods that would rely heavily on expert opinion and/or disease prevalence estimates that are difficult both to maintain outcome accuracy and to automate for regular updating. Alternative comparative approaches that would include improved collection of the non-pathology submission information to align with the minimum data set, specifically the inclusion of disease classification and/or reason for submission by the submitter, would provide greater opportunities for both syndrome classification and validation.

- Finally, the evaluation and analysis of the diagnostic outcomes from pathology submissions into meaningful clusters using MCA and hierarchical cluster analysis provided a reasonable way of ongoing syndrome validation. Based on multiple organ system diagnoses and comparable to other groups of clinical swine conditions, four viable pathology diagnoses clusters were established for syndrome validation, primarily respiratory (G1), primarily multisystemic (G2), primarily gastro intestinal (G4) and primarily ”other” (G3).

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Chapter 4: Syndrome classification using laboratory test requests and specimen types from porcine cases submitted for pathology diagnoses.

4.1 Introduction

Effective syndromic surveillance depends on the classification of pre-diagnostic indicators, such as clinical signs, pharmaceutical sales or laboratory test orders, into "syndromes" that can be used to indicate significant health events for timely and accurate assessment (Dórea et al, 2013; Hiller et al, 2013; Hoinville et al, 2013; Katz et al, 2011). As such, syndrome classification is an essential element in the development and implementation of the syndromic surveillance components of early warning surveillance systems (Hoinville et al, 2013; Ivanov et al, 2002; Reis and Mandl, 2004). The process of syndrome validation is also an important consideration that utilizes diagnostic outcomes or traditional surveillance methods to confirm syndrome accuracy (Betancourt et al, 2007; Chapman et al, 2005a; Ivanov et al, 2002; Kleinman and Abrams, 2008). Appropriate validation of syndrome classifications contributes utility and efficiency to a syndromic surveillance system by ensuring high sensitivities and positive predictive values when compared to real clusters of disease (Guasticchi et al, 2009; Kleinman and Abrams, 2008).

In public health, consideration has been given to two categories of syndrome classification based on the purposes of the syndromic methods (Katz et al, 2011). Syndromes may be "syndrome based" and targeted to specific disease conditions such as influenza-like illness (ILI), severe acute respiratory syndrome (SARS) or potential bioterrorism events such as anthrax. Significant increases in the occurrence of a targeted syndrome are similar in approach to hazard specific surveillance described for animal health in Chapter 1. This type of surveillance may lead more directly to mitigation and intervention, as the sensitivities may be sufficient to limit the need for further confirmation and investigation. Alternatively, syndromes may be "syndromic non-specific" and developed from multiple types of data sources with the intent of detecting unusual patterns in a broad range of health-related behaviours. The focus is therefore not on a specific risk event but rather on "at risk" health-related behaviours that may indicate a change in the health of the associated population and lead to additional investigation and confirmation. Syndrome classification in this category comes from preclinical data intended for other purposes, such as telehealth, ambulance dispatch, pharmaceutical sales, or laboratory test orders (Buehler et al, 2004; Heffernan et al, 2004; Katz et al, 2011; Kleinman and Abrams, 2008; Sintchenko and Gallego, 2009). For the purposes of public health,

veterinary medical records are also placed in this category (Katz et al, 2011). The disadvantage of the syndrome non-specific category, compared to the syndrome based approach, is that it may be less sensitive to known risk events such as ILI, especially if multiple data types (e.g. emergency department, laboratory, pharmacy) are not combined in the surveillance methods. However, the syndrome non-specific category has the advantage of identifying emerging or changing diseases, because all cases from all data types involved for the population under surveillance are included. In addition, conducting syndrome classification under a non-disease specific purpose adapts to different data types and can better utilize semi-structured data or free text (Dórea et al, 2013; Katz et al, 2011). Generally, syndromic surveillance for use in animal health has focused on methods that fit the "syndromic non-specific" category because (a) the purposes are often to enhance early warning surveillance for emerging or re-emerging diseases, and (b) the approach is more amendable to the inclusion of the multiple data types and data structures typically found in veterinary medicine information systems (Dórea et al, 2011; Dupuy et al, 2013b; Gibbens et al, 2008; Hoinville et al, 2013). A recent inventory of veterinary syndromic surveillance initiatives in Europe identified 25 syndromic surveillance systems either implemented or under development (Dupuy et al, 2013a). Consistent with the purposes of the "syndrome non-specific" category, the majority of the initiatives had multiple surveillance objectives for general animal health, used data collected for other purposes, included multiple data sources and had data structures with either internal disease coding or no coding at all.

In both public and animal health, the expanding capabilities of basic computing systems, plus increased volume and scope of electronic data capture of health information have greatly improved syndrome classification from pre-diagnostic data collected for other purposes. Rule-based methods to classify syndromes in public health are common as they have had excellent sensitivity and predictive values when validated, and are easily understood among collaborating health experts (Betancourt et al, 2007; Farkas and Szarvas, 2008; Heffernan et al, 2004; Ivanov et al, 2002; Reis and Mandl, 2004). However, considerable effort is required to automate rule-based methods, as they require extensive mapping of pre-diagnostic indicators and frequent domain expert review to maintain significance (Buehler et al, 2004; Farkas and Szarvas, 2008; Sosin and DeThomasis, 2004). To this end, public health has benefited from standardized disease nomenclature, such the *International Classification of Disease* coding, and

from the multiple collaborations among many domain experts in reviewing syndrome classification rules (Betancourt et al, 2007; Buehler et al, 2004). To take advantage of the volume of available data and to effectively utilize standardized disease nomenclature across large data sets, syndrome classification has expanded to include more advanced machine learning methods; Naive Bayes classifiers, Decision trees and others have been especially useful when coding free text or semi-structured data in large data sets across multiple sources to standardized nomenclature (Chapman et al, 2005a; Dórea et al, 2013; Dupuy et al, 2013a; Reis and Mandl, 2004). In animal health, the limited application of standardized nomenclature, the lower volume of data and the difficulty in maintaining expert review have made the long-term application of rule-based methods more difficult. There are some animal health surveillance systems, such as the United Kingdom's Veterinary Investigation Diagnosis Analysis system (VIDA) and Purdue University's National Companion Animal Surveillance Program (NCASP), that have greater success because these systems have established linkages between multiple data sources, routine expert review and analysis, data standardization and ongoing validation (Gibbens et al, 2008; Glickman et al, 2006; Paiba et al, 2007). Many other animal health data sources, such as diagnostic laboratories and abattoir inspection systems, have established nomenclature with resident domain expertise and as such should be amendable to either method. However, these sources also have considerable free text or semi-structured data types, have limitations in terms of expert resources for syndrome review and require a considerable degree of automation. For syndrome classification in animal health, these limitations make the use of advanced machine learning methods appealing, either alone or in combination with more traditional rule-based approaches (Dórea et al, 2013; Dupuy et al, 2013b; Farkas and Szarvas, 2008).

Validation of syndromic surveillance provides assurance of meaningful, representative approaches of "at risk" populations or the capacity to detect true biological events. The sensitivity, specificity and predictive values of early warning surveillance are important to detect significant disease events in a timely and accurate fashion while ensuring that limited resources are not expended on investigating too many false alarms (Bourgeois et al, 2006; Bravata et al, 2004; Katz et al, 2011; Kleinman and Abrams, 2008; van den Wijngaard et al, 2008). Validation of syndromes is a critical component of syndrome classification and requires an alternative high quality data component for comparison; data from traditional surveillance

methods, from diagnostic outcomes (e.g. laboratory results, case discharge codes) or from domain expert classification (Chapman et al, 2005b; Dórea et al, 2013; Hiller et al, 2013; Ivanov et al, 2002). The data may be collected retrospectively from the same sources through random allocation into training and test subsets or may involve prospective studies where representative subsets are further analyzed at time of collection (Bourgeois et al, 2006; Kashiouris et al, 2013; van den Wijngaard et al, 2008). Syndrome validation may use a variety of analytical methods, including accuracy measures (e.g. F-score), agreement metrics (e.g. kappa measures), general linear models (GLM), general linear mixed models (GLMM), and analysis of variance (ANOVA) (Kleinman and Abrams, 2008; Leal and Laupland, 2008). In animal health syndromic surveillance, the use of validation methods has not progressed to the level of those in public health. Many animal health syndromic surveillance systems have not been operating long enough to accumulate syndromic surveillance data from multiple sources for validation. In addition, the complex syndrome classification methods required for the disparate data types and structures make validation more difficult (Dórea et al, 2011; Dupuy et al, 2013a). A survey of veterinary syndromic surveillance systems in Europe did not specifically report validation methods used in the 27 systems that were represented by respondents. Epidemiologists in the VIDA system conducted a form of verification and investigation of the system's laboratory diagnostic coding through additional analysis of non-randomly assigned cases (Hyder et al, 2011; Kosmider et al, 2011). Amezcua compared syndrome counts from a practitioner based swine surveillance system with counts of laboratory cases using negative binomial regression (Amezcua et al, 2013). The method evaluated the impact of external factors, such as season and year. Dórea compared machine learning and rule-based syndrome classification methods from animal health laboratory submissions using accuracy measures but noted that further validation would be beneficial (Dórea et al, 2013).

The purpose of this chapter was to classify meaningful syndrome variables from specimen types and test requests from porcine cases submitted for diagnostic pathology and to assess their utility for syndromic surveillance. The approach was to first identify patterns of test requests and specimen types that cluster together in the data and utilize these clusters as "syndromes". Second, the approach was to estimate the ability to detect significant disease by using the syndromes as variables to predict pathology diagnoses for the submitted cases. The syndrome clusters were identified using agglomerative hierarchical cluster methods applied to

the case submission data within the Laboratory Information Management System (LIMS). A multinomial regression model was used to estimate specific syndrome prediction of diagnostic outcomes for cases previously assigned to one of four possible pathology diagnoses. Each pathology case was previously clustered into one of these four possible pathology outcomes based on organ system involvement (Chapter 3).

4.2 Methods

4.2.1 Data description

The data source was the Veterinary Diagnostic Services (VDS) LIMS described in Chapter 3. The LIMS data structure is based on observations that are individual results for each test request / specimen type combination. Each pathology case therefore has multiple observations. However, the primary diagnoses and the additional diagnoses were assigned at the case level, not the test request level. Tests and specimens submitted to VDS are automatically coded when entered into the LIMS, using a coding system specific to the database. At the case level, the number of unique test requests and specimen types available in the data set were determined and the case counts of where each occurred at least once were calculated. The most common test requests and specimen types were selected based on a wide range of conditions and body systems covered, as well as frequency of use. Test requests that represented post diagnostic follow up at external reference laboratories were excluded as the pathology diagnoses would have already been determined (e.g. virus sequencing).

Each pathology case was previously classified based on the primary and additional diagnoses by the attending pathologist. The classification was conducted through Multiple Correspondence Analysis (MCA) and the diagnoses were grouped into one of four outcomes based on the primary organ system (OS) involved: primarily Respiratory (G1 from Chapter 3), primarily Multisystemic (G2 from Chapter 3), primarily Gastro-Intestinal (G4 from Chapter 3) and “Other” (G3 from Chapter 3).

4*.2.2 Syndrome Classification*

Syndrome classification was conducted using agglomerative hierarchical cluster analysis to select, extract and reduce the test requests and specimen types into syndrome components. Agglomerative hierarchical clustering measures the similarity (or dissimilarity) between sets of observations, generated from pair-wise distances among observations (Hastie et al. 2001).

Agglomerative means the clusters are formed in a "bottom up" process, starting from a single pair of observations. Hierarchical clustering in binary data can be explored using different similarity or dissimilarity measures to weight the pair-wise distances among observations. Matrices of the different measures are generated across binary variables and the distances between groups are measured using the following linkages: nearest neighbor(single), furthest neighbor (complete), group average, weighted average, group median, group centroid and minimum variance (Ward's linkage) (Hastie et al 2001). The technique has been used to evaluate a variety of biological data, such as cancer diagnoses and DNA analyses (Hastie et al 2001). All matrices and cluster analyses were conducted using multivariate statistical tools in the statistical software (*Stata Statistical Software: Release 11, 2009*).

In order to classify syndromes based on test requests and specimen types, individual binary variables representing each test request and specimen type were mapped to the case level pathology submission data using internal coding. Syndromes were generated by first evaluating the relationships between the test and specimen variables. Matrices were generated using 11 different binary dissimilarity measures. The key measures used were those that provided greater weight to similarities (variables equal to one). Hierarchical cluster analysis was conducted on each matrix using 5 different cluster linkages; simple, complete, average, weighted average and Ward. The Duda-Hart and Calinski-Harabasz cluster stopping rules were used to indicate the most significant number of natural clusters in each analysis. The selection of clusters was based on natural clusters within the data that had the greatest agreement between the two stopping rules. The natural clusters with the greatest agreement were tabulated and compared across all measures and linkages. The natural clusters were then evaluated for relevancy and consistency to ensure they represented real world clinical and pathology scenarios. The most relevant and consistent clusters were assigned to syndromes and mapped in the statistical software to individual cases using the internal LIMS coding. Each case had to have a minimum of one test request from the syndrome in order to be assigned a syndrome. Since the intent of the study was to determine the predictability of each syndrome for pathology diagnoses across all cases, individual cases could be assigned more than one syndrome.

4.2.3 Multinomial logistic regression

The purpose of the syndrome classification model was to estimate which syndromes had the most significant predictability for the four diagnostic outcomes. The full dataset was randomly allocated into a training dataset (60% or 2835 cases) for syndrome prediction and a test dataset (40% or 1891 cases) for syndrome validation. The statistical analyses were conducted using Stata (*Stata Statistical Software: Release 11, 2009*). A multinomial logistic regression model was used because the outcome variable consisted of four OS diagnoses. The data were considered nominal as each diagnostic outcome was considered independent of the other categories and no assumptions could be made about the order of the outcome categories. The ""Other"" OS group was considered the baseline category as it represented all other organ system diagnoses.

Variables representing influential factors on the number of cases submitted and variables representing factors that may correlate with the selection of tests or specimen types were also included in the regression model. The evaluation and analysis of the data source in Chapter 3 noted considerable variation in the number of submissions over the time-frame of the dataset. In order to control for case submission variation over time within the data set three different measures were used; case submission dates, year of submission and equal time periods were separately evaluated. The equal time period was a categorical variable with three 25-month time periods, separating the data set into equal parts. The effects of seasonal quarters (Q1 represented January – March, Q2: April – June, Q3: July – September, Q4: October – December) and day of week were also considered, and included in the model as categorical variables. It was considered likely that the number of tests requested and number of specimen types per case could correlate with different diagnoses and the selection of specific syndromes, as certain syndrome clusters would naturally include more specimen types and test requests. Both factors were therefore included in the model as continuous variables.

Model development was carried out using a step-wise approach. Individual variables were first assessed for significance through univariate multinomial models using Wald and likelihood ratio tests. Full and reduced models were compared using likelihood ratio tests (if one model was nested in another), Akaike's information criteria (AIC) and the Bayesian information criteria (BIC) (Dohoo et al. 2009). Model diagnostics were conducted through a variety of means. The

model fit was evaluated using a generalized Hosmer – Lemeshow goodness of fit test for multinomial logistic regression models in groups of 30, 20, 15, 12 and 10 (Fagerland and Hosmer, 2012). Regression diagnostics through individual logistic regression models were used to assess outliers and observations with undue influence (Dohoo et al 2009).

The predictive power of the model was assessed using difference in predictions adjusted for its standard error (Stata Press 2009). Sensitivity and specificity for each outcome were also assessed at different cut points.

4.2.4 Syndrome validation

The purpose of the syndrome validation step was to determine if the predictive model developed through the training data set had similar predictions in the test data set. Two measures were used to validate the predictive ability of each syndrome between the two data sets. The relative risk ratios for each syndrome between pairs of OS diagnoses were calculated (Long and Freese utilities) and compared. The effect that the presence of each syndrome had on the probability of observing an OS diagnosis (adjusted predictions) were calculated and compared using the post estimation margins command in the statistical software. Finally, the margins calculations were used compare the predictive probabilities of groups of syndromes at their set values. This approach evaluated which syndromes grouped together to predict an OS diagnosis as well as validated the syndrome groups between the two data sets.

4.3 Results

4.3.1 Data Description

The data set for syndrome classification contained 4726 pathology cases with 49977 unique test requests and specimens submitted for use in syndrome classification: The number of unique test requests per case across all cases was 28434. The median number of test requests per case was 6 with a 25^{th} percentile of 4, a 75^{th} percentile of 8 and a range of 1 to 17. The 30 most common test requests occurred at a cut point of 0.3% of cases and represented 98% of the total unique test requests. Test requests below this value were either extremely rare (e.g. lead toxicity) or were dependent on other more frequent tests. The number of unique specimen types per case across all cases was 21543. The median number of specimen types submitted per case was 4 with a 25^{th} percentile of 3, a 75^{th} percentile of 6 and a range of 1 to 13. The 34 most common specimen types occurred at a cut point of 0.3% of cases and

represented 97% of the total unique specimen types submitted. The specimen types below this value tended to be rare and/or non-specific (e.g. limb, feed sample). The data set included 1408 submission days, averaging 3.36 cases per submission day. The 25-month time periods included 30% of cases in the first period, 38% in the second and 32% in the third. For seasonal quarters, there were 29% of cases in Season 1, 24% in Season 2, 19% in Season 3 and 28% in Season 4. For day of week, 15% of cases were submitted on Monday, 22% on Tuesday, 23% on Wednesday, 21% on Thursday and 19% on Friday. The proportion of organ system (OS) diagnoses in the full data set was 44.1% Respiratory, 20.4% Multisystemic, 19.9% Gastrointestinal and 15.7% "Other".

4.3.2 Syndrome Classification

From cases with pathology diagnoses, the 30 most common test requests and the 34 most common specimens submitted were included in the hierarchical cluster analysis. From an earlier systematic review of public health syndromic surveillance it was estimated that any number greater than 15 syndromes would not be reasonable for surveillance purposes (Chapter 2). The stopping rules were utilized to estimate the most relevant clusters within each analysis. Typically, groups of 5, 7, 9, 10 and 12 clusters were the preferred sizes across most measures and analyses. Measures that weighted agreement (variables equal to 1) appeared to produce the most reasonable clusters. These measures included Jaccard, Dice, Yule and Anderberg. Figures 1a, 1b and 1c are examples of the types of dendrograms and group descriptions that resulted from the cluster analysis with different dissimilarity measures and linkages. The branches of the dendrograms indicate which groups have greater similarity (lower dissimilarity measure) and therefore are more likely to cluster together. Likewise, the higher the dissimilarity measure, the greater the distance between branches, the less similarity between groups.

There was not perfect agreement across all hierarchical cluster analyses. However, several distinct groups of test requests and specimens submitted were consistent across multiple measures. Several of these distinct groups are also consistent with typical clinical diagnostic approaches to swine diseases (Pedersen et al, 2010; Zimmerman et al. 2012). Test requests and specimen types for specific clinical conditions related to porcine reproductive disease (Figure 1a, Group 9; Figure 1b, Group 10; Figure 1c, Group 12), porcine respiratory disease (Figure 1a, Group 5; Figure 1b, Group 4; Figure 1c, Group 3), neonatal diarrhea (Figure 1a,

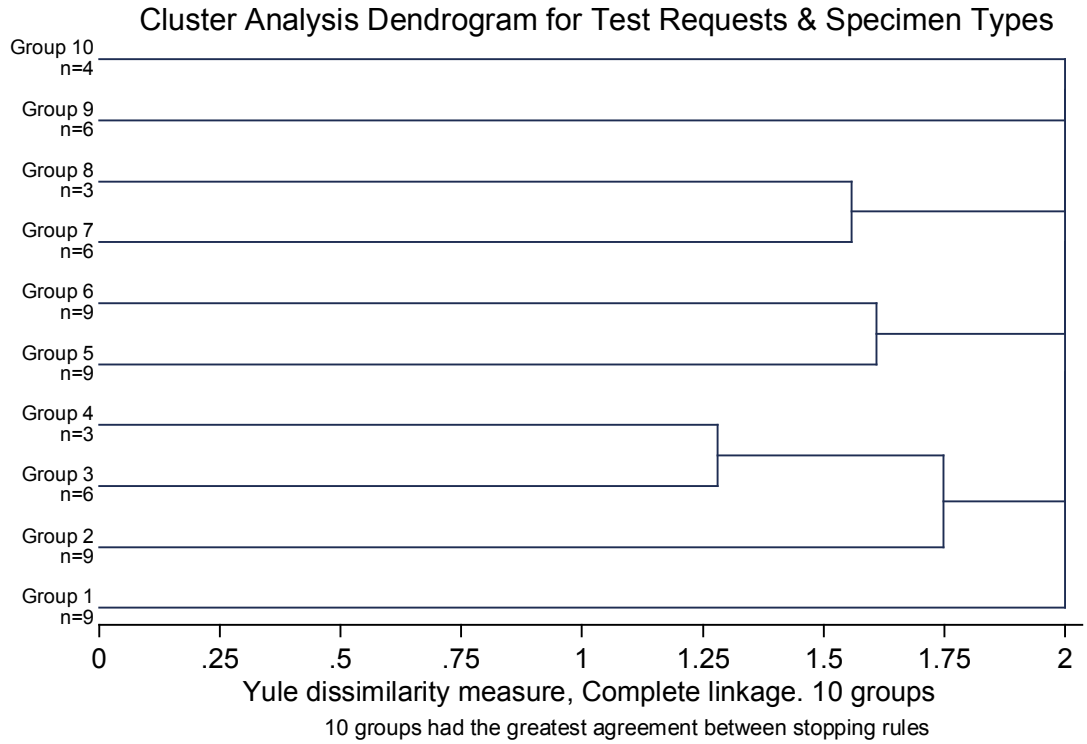
Group 1&2; Figure 1b, Group 1; Figure 1c, Group 5&6), chronic diarrhea in grow/finish pigs (Figure 1a, Group 3; Figure 1b, Group 3; Figure 1c, Group 7) and severe lameness in growing pigs (Figure 1a Group 7; Figure 1b, Group 6; Figure 1c, Group 8) consistently grouped across multiple measures. Furthermore, branches with groups of greater clinical similarity also clustered in the analysis. For example, Groups 4-6 in Figure 1a had a low dissimilarity measure (<1.5) and were consistent with gastrointestinal conditions.

Certain non-specific groups of test requests and specimen types, such as carcass, fixed tissue, necropsy, histopathology and bacterial cultures did not consistently group in distinct clusters across the different measures. Specific test requests for common endemic and multisystemic swine diseases such as porcine reproductive and respiratory syndrome (PRRS) and porcine circovirus associated disease (PCVAD) tended not to form any distinct group or groups. However, there was a greater tendency for the test requests for these two diseases to cluster with respiratory conditions.

The cluster analyses were reviewed for relevance, both from a clinical and surveillance perspective. Based on this review, the 14 syndromes listed in Table 1 were selected for prediction. Histology, aerobic culture and fixed tissues were considered nonspecific and common across all pathology submissions. As a result these were considered more as confounders than predictive variables. Necropsy was also held distinct as a confounder because it differentiated necropsies conducted by veterinary pathologists in contrast to field necropsies conducted by clinical veterinarians. The respiratory category was adjusted to include respiratory specific specimens and tests. PRRS and PCVAD were considered significant swine diseases with a variety of clinical and pathology presentations (Carman et al, 2008; Madec et al, 2008; Young et al, 2010) and therefore were assigned specific syndromes based on test requests. Through the cluster analyses, lymphatic tissue submissions did not consistently cluster with any one group of test requests or other specimen types.

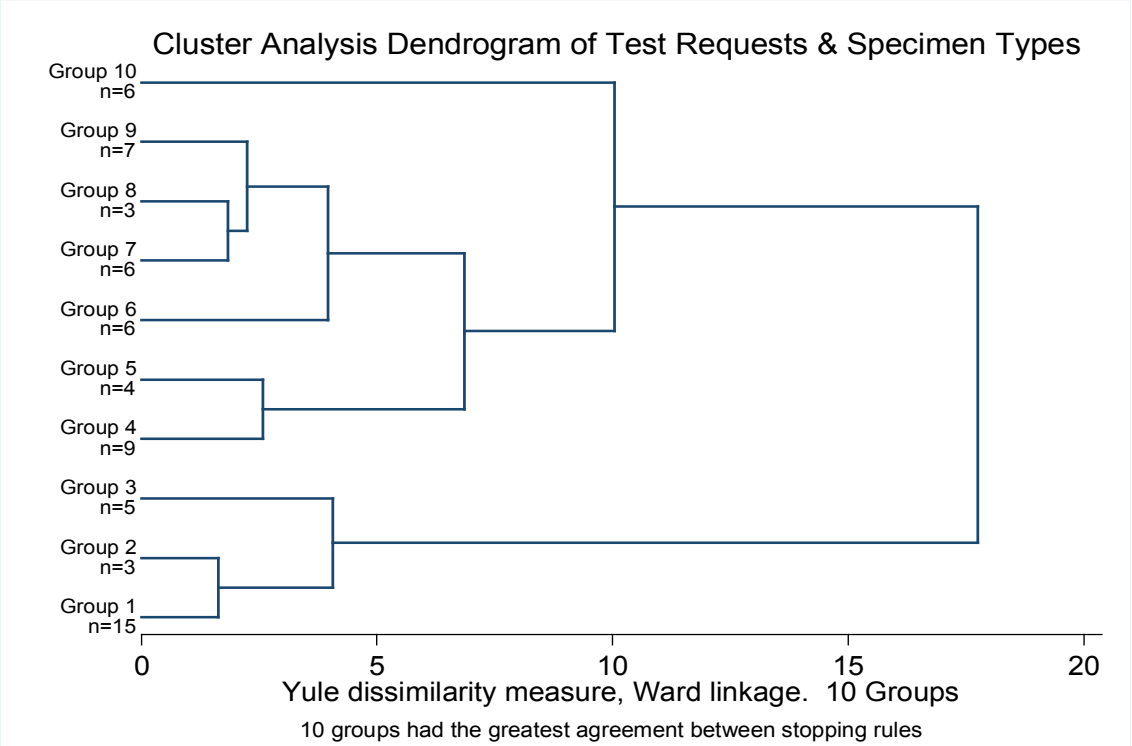
While some specific lymphoid tissues may be submitted for specific diagnoses, lymphatic tissue is generally included in most submissions because of its use in diagnosing many different diseases. For similar reasons to histopathology/culture and necropsy, Lymph node submissions were assigned to a separate syndrome as they may be seen as confounding of other

Figure 1a: A 10 syndrome group outcomes from cluster analysis on 30 test requests and 34 specimen submissions, using Yule dissimilarity measure and complete linkage.



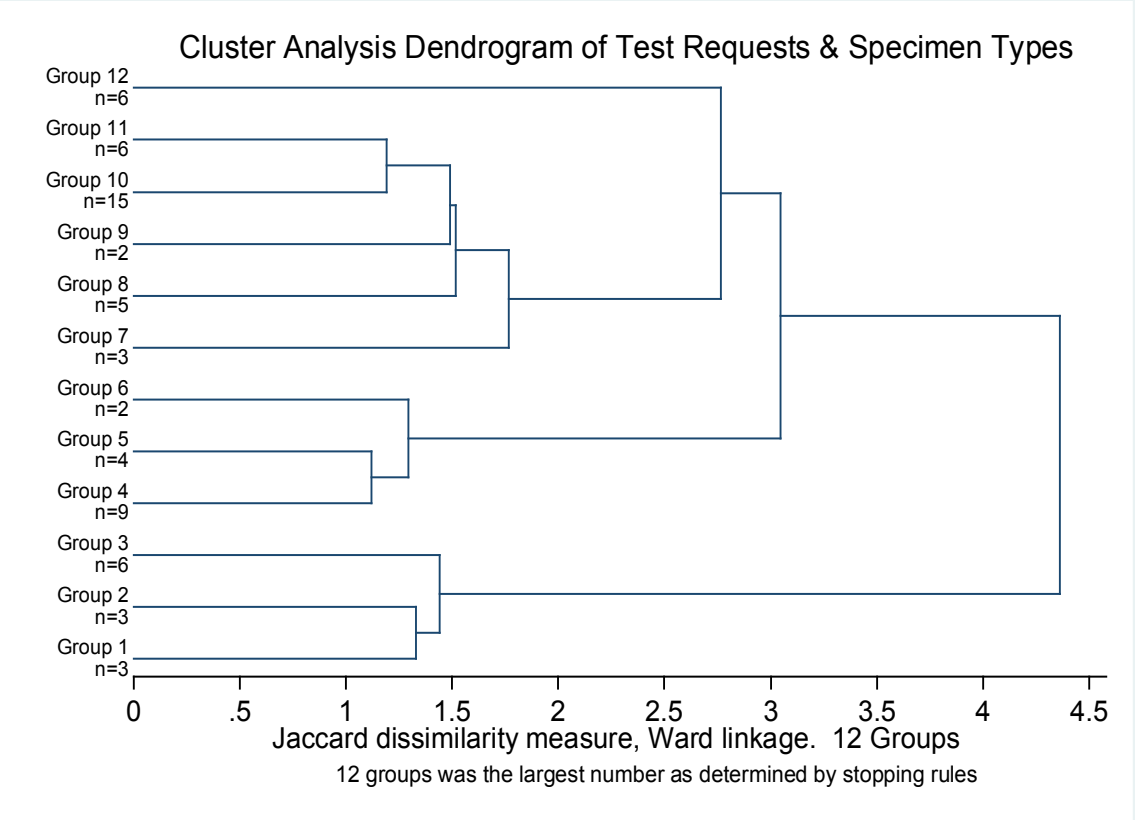
Group 10	Lymph node (Not specified), tonsil, brain swab, skin
Group 9	FAT for Porcine parvovirus. Fetal necropsy, fetus, fetal tissue, fetal stomach contents, female reproductive tissue
Group 8	PCR for Cytomegalovirus. Nasal swab, body fluid (Not specified)
Group 7	PCR for *M. Hyosynoviae*. Gram stain, joint tissue, synovial membrane, joint swab
Group 6	*Streptococcus suis* typing. Carcass, neurologic tissue, heart, kidney, spleen, pooled liver/spleen, pooled lung/spleen, pooled lung/tonsil
Group 5	PCR tests for swine influenza (H3N2 & H1N1), *M hyopneumoniae*, PRRS & PCV, Genotyping for PCV or PRRS. Lung tissue
Group 4	Micromineral analysis. Liver tissue, serum
Group 3	*E. coli* K88 serotyping, PCR tests for *B. hyodysenteriae, B. pilosicoli, L. Intracellularis*. Intestine (Not specified), fixed tissue
Group 2	Necropsy, aerobic culture, anaerobic culture, PCR for *C. perfringes*. Live animal submission, Small intestine, Culture tube, smear
Group 1	Histology, FAT for Porcine corona virus (Transmissible Gastroenteritis), ELISA for *C difficile,* Serotyping for *E coli,* Electron Microscopy, Coccidial smear. Large intestine, feces(fixed)

Figure 1b: A 10 syndrome group outcome from cluster analysis of 30 test requests and 34 specimen submission variables, using Yule dissimilarity measure and Ward linkage.



Group 10	FAT for Porcine parvovirus. Fetal necropsy, fetus, fetal tissue, fetal stomach contents, female reproductive tissue
Group 9	*Streptococcus suis* typing. Carcass, neurologic tissue, heart, pooled liver/spleen, pooled lung/tonsil, brain swab
Group 8	Lymph node (Not specified), skin
Group 7	Serotyping for *E coli,* Micromineral analysis. Liver tissue, kidney tissue, spleen tissue, pooled lung/spleen.
Group 6	PCR for *M. Hyosynoviae*. Gram stain, body fluid (Not specified), joint tissue, synovial membrane, joint swab
Group 5	PCR for Cytomegalovirus. Nasal swab, serum
Group 4	PCR tests for swine influenza (H3N2 & H1N1), *M hyopneumoniae*, PRRS & PCV, Genotyping for PCV or PRRS. Lung tissue
Group 3	PCR tests for *B. hyodysenteriae, B. pilosicoli, L. Intracellularis*. Intestine (Not specified), fixed tissue
Group 2	Necropsy, Live animal submission, feces
Group 1	Histology, FAT for Porcine corona virus (Transmissible Gastroenteritis), ELISA for *C difficile,* PCR for *C. perfringes, E. coli* K88 serotyping, Electron Microscopy, Coccidial smear, smear. Large intestine, feces(fixed), aerobic culture, anaerobic culture, Small intestine, Culture tube.

Figure 1c: A 12 syndrome group outcome from cluster analysis of 30 test request and 34 specimen submission variables, using the Jaccard dissimilarity measure and Ward linkage.



Group 12	FAT for Porcine parvovirus, Fetal necropsy. Fetus, fetal tissue, fetal stomach contents, female reproductive tissue
Group 11	*Streptococcus suis* typing. Neurologic tissue, brain swab, heart, spleen, kidney
Group 10	Genotyping for PCV or PRRS. Serotyping for *E coli,* PCR for Cytomegalovirus. Feces, lymph node (Not specified), pooled liver/spleen, pooled lung/spleen, tissue (Not specified), Body fluid (Not specified) Nasal swab, tonsil, serum, skin
Group 9	Micromineral analysis. Liver tissue.
Group 8	PCR for *M. hyosynoviae*. Gram stain, joint tissue, synovial membrane, joint swab
Group 7	PCR tests for *B. hyodysenteriae, B. pilosicoli, L. Intracellularis*
Group 6	PCR for *C. perfringes*. Culture tube
Group 5	FAT for Porcine corona virus (Transmissible Gastroenteritis). Fecal smear
Group 4	Electron Microscopy, anaerobic culture, ELISA for *C difficile*, *E. coli* K88 serotyping. Feces (fixed), coccidial smear. Live animal submission, all intestinal tissue
Group 3	PCR tests for swine influenza (H3N2 & H1N1), *M hyopneumoniae*, PRRS & PCV. Lung tissue
Group 2	Necropsy. Carcass, pooled lung/tonsil
Group 1	Histology, aerobic culture. Fixed tissue

Table 1: List of Syndromes	
Syndrome	Description
Histology/Culture[1]	Specimens submitted included all fixed tissue not specifically identified Tests requests were for histology and/or anaerobic culture
Necropsy[2]	Full necropsy conducted by pathologists at laboratory on submitted carcasses
Respiratory	Specimen submitted was Lung tissue Test request were PCR tests for *Mycoplasma hyopneumoniae*, Swine Influenza H1N1 or Swine Influenza H3N2
PRRS	Test requests were PCR tests for PRRS, and/or sequencing / genotyping for PRRS virus strains.
PCV	PCR test requests for PCVAD, +/- genotyping for PCV
Lymph Node Submission	Specimens submitted were for lymph node tissue, including tonsil and lymph nodes pooled with lung.
GI syndrome 1	Specimens included were live animal submissions[2] , all intestinal submissions, plus formalin fixed fecal samples. Test requests included electron microscopy, parasite detection (especially for coccidiosis), anaerobic culture, and specific tests for Porcine Coronavirus (Transmissible Gastroenteritis), *Clostridium difficile, Clostridium perfringes,* and *Escherichia coli* K88
GI syndrome 2	Subset of GI syndrome 1 where cases included test requests for *Lawsonia intracellularis, Brachyspira hyodysenteriae* and/or *Brachyspira pilosicoli*
Reproduction	Specimens included are fetal tissues plus female reproductive tissue Test request was for porcine parvovirus
Joint	Specimens included are any joint tissue, including synovial membranes and joint swabs. Test requests were for PCR tests for *Mycoplasma hyosynoviae* and gram stains
Hepatic	Specimen type included liver Test requests were for micronutrient analysis
Circulatory	Specimen type only; Heart, kidney, spleen
Neurologic	Specimens included any neurologic tissue including spinal cord, meninges and swabs of brain Test requests included *Streptococcus suis* typing
Rhinitis	Specimen type included nasal swabs Test requests included PCR test for Cytomegalovirus

1. Histology / culture may be conducted on tissues submitted from field necropsies or from full necropsies conducted by pathologists at the laboratory.
2. Necropsy excluded live animals submitted to the laboratory for euthanasia and subsequent necropsy. These cases clustered more with the GI syndromes.

syndromes. GI syndrome 1, Reproduction, Joint, Hepatic, Circulatory and Neurologic were syndromes that consistently clustered through the analysis and individually had strong clinical relevance. GI Syndrome 2 and Rhinitis were seen as more specific subsets of GI Syndrome 1 and Respiratory, respectively. Through the cluster analyses, both of these tended to cluster

either within or adjacent to their “parent” syndromes. Clinically each syndrome represents specific disease conditions within their “parent” category; GI Syndrome 2 represents test requests and specimen types expected for chronic diarrhea in grow/finish pigs. Rhinitis represents severe upper respiratory tract infections in nursing piglets.

Further comparisons and linkages of the 14 syndromes were conducted through cross tabulations of cases in full data set. The most frequently occurring syndromes are listed in Table 2. The table also includes the percentage of each syndrome’s cases that also have more commonly occurring syndrome, if the percentage exceeded 50%. The cross tabulations confirmed Histology/Culture as a very common, nonspecific component of all submissions, since it occurred in almost 98% of all pathology cases. Laboratory necropsies conducted on submitted carcasses were associated with 51% of all cases and aligned with Histology/culture. 74% of lymph node submissions occurred together with necropsy submissions, indicating that lymph node submissions mostly occurred when a pathologist conducted the necropsy.

The respiratory syndrome was the most frequent disease related syndrome and was associated with 67% of all submissions. Many cases with the respiratory syndrome also had other syndromes such as PCV, PRRS, Circulatory and Rhinitis. PCV and PRRS syndromes were strongly linked to the more general respiratory syndrome as well as to each other; 43% of all cases included both PCV and PRRS syndromes, and over 90% of the cases with either or both of these syndromes also included the respiratory syndrome. The cross tabulation confirmed that the rhinitis syndrome was primarily a subset of respiratory with only 4% of the cases with the syndrome not also having the respiratory syndrome. The rhinitis syndrome occurred in 5% of all cases and 7% of all respiratory syndrome cases.

Cases with the GI syndromes did not frequently include other syndromes. The cross tabulation confirmed the secondary, more specific GI syndrome was a subset of the primary GI syndrome with 100% correlation. The GI Syndrome 2 was included in approximately 13% of all cases and in 26% of cases that included GI Syndrome 1.

The other syndromes (Joint, Neurologic, Reproduction and Hepatic) ranged between 12% and 5% of all cases. The cross tabulation supported the cluster results in that these syndromes also

did not frequently occur together. In particular, the reproductive syndrome and the joint syndrome remained distinct.

Table 2: Frequency of syndromes variables across all pathology cases

Syndrome	% of all submissions	Occurrence with more frequent syndromes	% of syndrome cases with more frequent syndrome
Histology/Culture	97.9%	----------	-------
Respiratory	67.0%	----------	-------
PRRS	54.1%	Respiratory	92.1%
GI Syndrome 1	52.6%	----------	-------
Necropsy	51.0%	Histology/Culture	96.5%
PCV	49.1%	Respiratory	90.2%
Circulatory	34.5%	Respiratory	87.7%
Lymph Node	34.0%	Necropsy	74.2%

4.3.3 Multinomial Logistic Regression

Using random allocation, the full data set was divided once into a training data set of 2835 cases and a test data set of 1891 cases for syndrome prediction. Table 3 contains the results from the multinomial logistic regression model with "Other" OS diagnosis as the baseline. Syndromes with significant positive coefficients (positive influence) for an OS diagnoses indicated that the presence of the syndrome in a case increased the probability of that OS diagnoses actually occurring in the case. Likewise, syndromes with significant negative coefficients (negative influence) for an OS diagnoses indicated that the presence of the syndrome decreased the probability of that OS diagnoses actually occurring in the case. The likelihood ratio and Wald tests were used to assess the overall significance of each syndrome and confounder in the model (Dohoo et al 2009). Rhinitis was the only syndrome that did not have overall significance in the model. However, this syndrome was retained because it improved overall model fit.

Unconditional associations were evaluated for all variables. All syndrome variables were significant in univariate multinomial logistic regression models with the "Other" OS diagnosis as the baseline. This indicated that each individual variable predicted one or more of the outcomes significantly better than the null model. Long and Freese utilities were further used in the univariate models to determine if pairs of outcome diagnoses were not significantly different from each other, through Wald and Likelihood ratio tests (Fagerland et al, 2008). All four diagnosis outcomes were significantly different from each other in univariate models with

Respiratory, Reproduction, PRRS, PCV and Circulatory syndromes as the univariate predictors. Two of the four outcomes were not significantly different from each other in two sets of univariate models; Respiratory and Multisystemic diagnoses were not significantly different with Histology/Culture, Necropsy, GI1, GI 2, Lymph node, or Joint syndromes as the predictors. Multisystemic and "Other" diagnoses were not significantly different with Neurologic syndrome as the predictor. Finally, two univariate models with either Hepatic or Rhinitis syndromes as the predictors had three of the four outcomes (Multisystemic, Gastrointestinal and "Other") not significantly different from each other.

Season was significant, but year and day of the week were not. The 25-month time period and submission date were significant. However the univariate model with the 25-month time period variable had a better fit to the data. This variable was used in the full model to represent variations in submissions over time.

The number of unique specimen types per case and number of unique test requests per case had overall significance in univariate multinomial models with "Other" OS diagnosis as the base outcome. The number of test requests remained significant in pair wise comparisons between respiratory, gastrointestinal and multisystemic OS diagnoses. However, the number of specimen types per case was not significant between these OS diagnoses. This is reflected in the greater change in probability of the OS diagnoses observed for each outcome with the number of test requests (Figure 2). There is a substantial change in probability over the number of test requests than over the number of specimen types, especially for Respiratory and "Other" diagnoses. Only the probability of "Other" diagnosis appears to decrease significantly with an increase in the number of specimen types. The linearity of both continuous variables was assessed using simple logistic models for each outcome. Linearity could not be achieved across all outcomes, but was achieved for two or more outcomes by using a quadratic transformation corrected for colinearity by centering on the means. Categorical, polynomial and fractional polynomial transformations of both variables were also attempted. However, squared transformations centered on means had the best overall model fit.

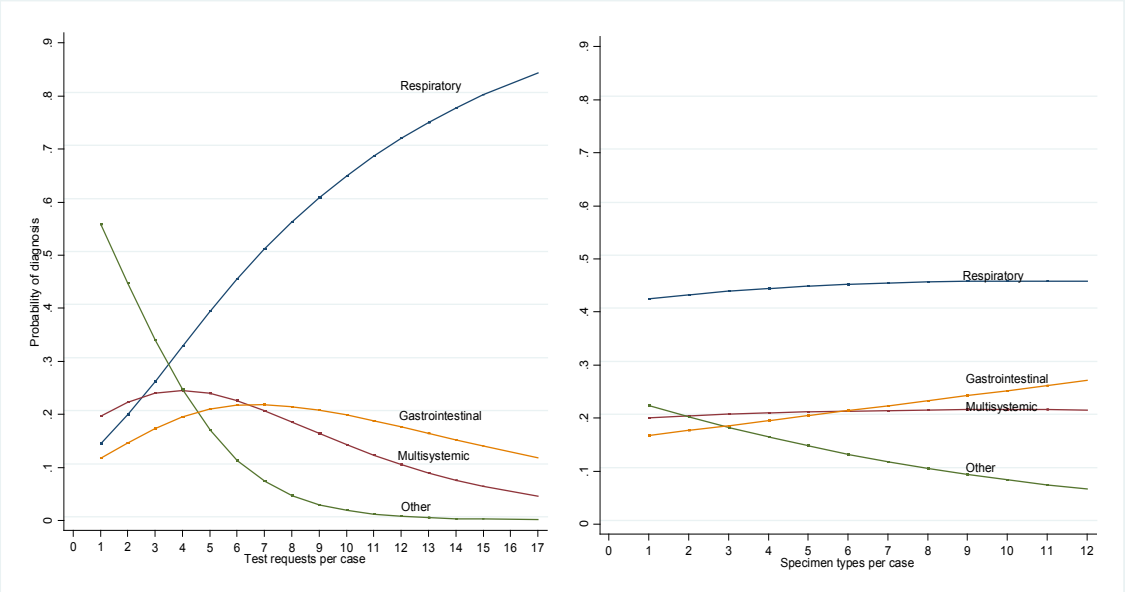
Table 3: Results from multinomial logistic regression model; "Other" diagnosis group and time period 1 are the reference categories. Coefficients with p value<0.05 or greater included.

	Respiratory Group			Multisystemic Group			Gastrointestinal Group		
Syndrome/Variable	Coef.	SE	95% CI	Coef.	SE	95% CI	Coef.	SE	95% CI
Respiratory	1.32**	0.19	0.94, 1.69	1.19**	0.20	0.80, 1.57	-1.05**	0.26	-1.55, -0.55
PRRS							-0.92**	0.32	-1.54, -0.30
PCV				0.46	0.23	0.01, 0.90	-0.92**	0.31	-1.52, -0.32
Rhinitis									
Gastrointestinal 1							2.62**	0.39	1.86, 3.37
Gastrointestinal 2	1.39*	0.54	0.33, 2.45	1.54*	0.55	0.46, 2.46	1.54*	0.55	0.46, 2.61
Circulatory				0.43	0.20	0.04, 0.83	-1.14**	0.29	-1.70, -0.57
Hepatic	-0.84**	0.26	-1.35, -0.33				-1.24**	0.38	-1.98, -0.50
Joint	-1.33**	0.21	-1.74, -0.92	-1.13**	0.22	-1.55, -0.70	-4.19**	0.49	-5.16, -3.22
Neurologic	-0.88**	0.20	-1.28, -0.48				-1.85**	0.39	-2.62, -1.07
Reproductive	-2.82**	0.32	-3.45, -2.18	-1.58**	0.31	-2.18, -0.98	-4.00**	0.87	-5.70, -2.31
Histology/Culture				1.21	0.57	0.10, 2.32	-2.80**	0.61	-4.00, -1.61
Necropsy (Pathologist)	-0.53*	0.19	-0.91, -0.16	-0.44*	0.20	-0.83, -0.06	-1.09**	0.26	-1.60, -0.57
Lymph Node	0.42	0.21	0.00, 0.84	0.72**	0.22	0.29, 1.15	-0.64	0.32	-1.27, -0.02
Period 2				0.54**	0.18	0.18, 0.90			
Period 3				0.46*	0.19	0.10, 0.83			
[t]Tests / Case	0.62**	0.07	0.49, 0.75	0.29**	0.07	0.16, 0.43	0.63**	0.08	0.46, 0.80
[t](Tests /Case)^2				0.04	0.02	0.01, 0.07			
[t]Specimens / Case				-0.17	0.09	-0.33, 0.00	0.34**	0.11	0.12, 0.56
[t](Specimens / Case)^2	0.05**	0.02	0.02, 0.09						
Intercept	1.16	0.67	-0.16, 2.47	-2.05*	0.76	-3.54, -0.57	4.71**	0.83	3.07, 6.34

* Coefficients with p values < 0.01, **Coefficients with p < 0.001.

t Counts of unique Test requests and specimen types per case have been centered on means and squared to adjust for linearity and colinearity.

Figure 2: Probability of organ system diagnoses by number of tests requested and specimen types per case.



Possible two-way interactions were determined from the data and subject area knowledge. Additionally, pairs of syndromes that a significant influence on the same OS diagnoses were also considered (e.g. neurologic and hepatic positively influence "Other" and Multisystemic OS diagnoses). The interaction terms evaluated were as follows: (i) Histology/culture and Necropsy submissions; (ii) GI syndrome 1 and 2; (iii) Respiratory and Rhinitis; (iv) Respiratory and PRRS; (v) Reproductive and PRRS; (vi) PCV and Lymph node submission, (vii) Neurologic and Hepatic; (viii) Neurologic and Circulatory; and, (ix) Hepatic and Circulatory. Two-way interactions were excluded from the final model because the interactions evaluated were not significant in the overall model and the models with interaction terms did not improve the fit of the model to the data. However, three interaction terms were significant, in each case for one of the four OS diagnoses;

- Including the interaction between the neurologic and hepatic syndromes further decreased the probability of a respiratory OS diagnosis when each occurred separately. The interaction of the two syndromes was positive indicating that when they occurred together, the probability of a respiratory OS diagnosis increased instead.
- The interaction of the PRRS and respiratory syndromes increased the probability of a respiratory OS diagnosis attributed to the respiratory syndrome alone. The interaction of the two syndromes was negative, indicating that when they occurred together, the

probability of the respiratory OS diagnosis decreased. The PRRS syndrome remained non-significant for respiratory OS diagnosis.

- The probability of Multisystemic OS diagnosis increased through the interaction of neurologic and circulatory syndromes. The interaction caused the neurological syndrome alone to decrease the probability of the diagnosis, while the circulatory syndrome alone no longer significantly predicted the diagnoses. Without the interaction term, the neurological syndrome did not significantly predict the multisystemic OS diagnosis while the circulatory syndrome significantly increased the probability of the diagnosis. The influence of the circulatory syndrome on the multisystemic diagnoses appears to be only when both syndromes are present in a case.

The expected and observed frequencies for all groups in the Hosmer – Lemeshow goodness of fit test were not significantly different, indicating the model fit the data. The quadratic terms used for number of test requests and specimen types were necessary to obtain model fit. Reduced models were significantly different than the final model, whereas full models improved over the final model only with the inclusion of rhinitis.

Outliers and observations with undue influence were evaluated through individual logistic regression models for each outcome as there are no comparable methods for multinomial regression models. The purpose was to identify what observations did not fit or had excessive influence on the multinomial model. Since the data set was a complete census of laboratory data and the overall purpose was to validate the predictability of syndromes, no observations were removed. Instead, the outliers and influential observations were further explored where possible to estimate reasons for the influence. 60-70 highly leveraged observations with only one test request and one specimen type submitted had influence on all four diagnostic outcomes. Approximately 60% of the cases had a necropsy submission where the pathologist made a diagnosis during the necropsy and no further testing was required. These were slightly more common in gastrointestinal OS diagnoses than the other three and corresponded to acute traumatic gastrointestinal events, such as stomach ulcers. The remaining 40% were single specimen types submitted for bacterial culture or histopathology and a diagnosis was

provided from the single test. These values may have impacted the overall fit of the multinomial model.

Outliers and influential observations with a gastrointestinal OS diagnosis: The multinomial model strongly supports the GI syndrome as the single predominant predictor for a gastrointestinal OS diagnosis. Cases that did not have a GI syndrome, do not have a syndrome selected nor had multiple syndromes selected, were the most pronounced outliers. A single predominant outlier was an individual case without a GI syndrome and with multiple other syndromes, including reproductive, respiratory, circulatory, neurologic, PRRS and PCV. The reproductive syndrome especially is a negative predictor for Gastrointestinal OS diagnosis (Table 3). Seven other cases did not have any syndromes selected. These were cases had been submitted for specific, rare test requests that were not included in any of the syndromes.

Outliers and influential observations with a respiratory OS diagnosis: The multinomial model indicates that the respiratory syndrome is a strong predictor for a respiratory OS diagnosis. Outliers included 17 cases with a respiratory OS diagnoses and without a respiratory syndrome. Many of these cases also included GI 1 and GI 2 syndromes, as well as circulatory or neurologic syndromes. Observations with leverage values included 6 cases with respiratory and rhinitis syndromes, but a multisystemic OS diagnosis. These cases tended to include PRRS and PCV syndromes as well, suggesting a true multisystemic involvement. Others included respiratory cases without a respiratory syndrome and/or a strong negative predictor such as reproductive or GI 1 or MS with a respiratory syndrome. Both sets of observations could have impacted the multinomial model fit, but the overall number of cases was low.

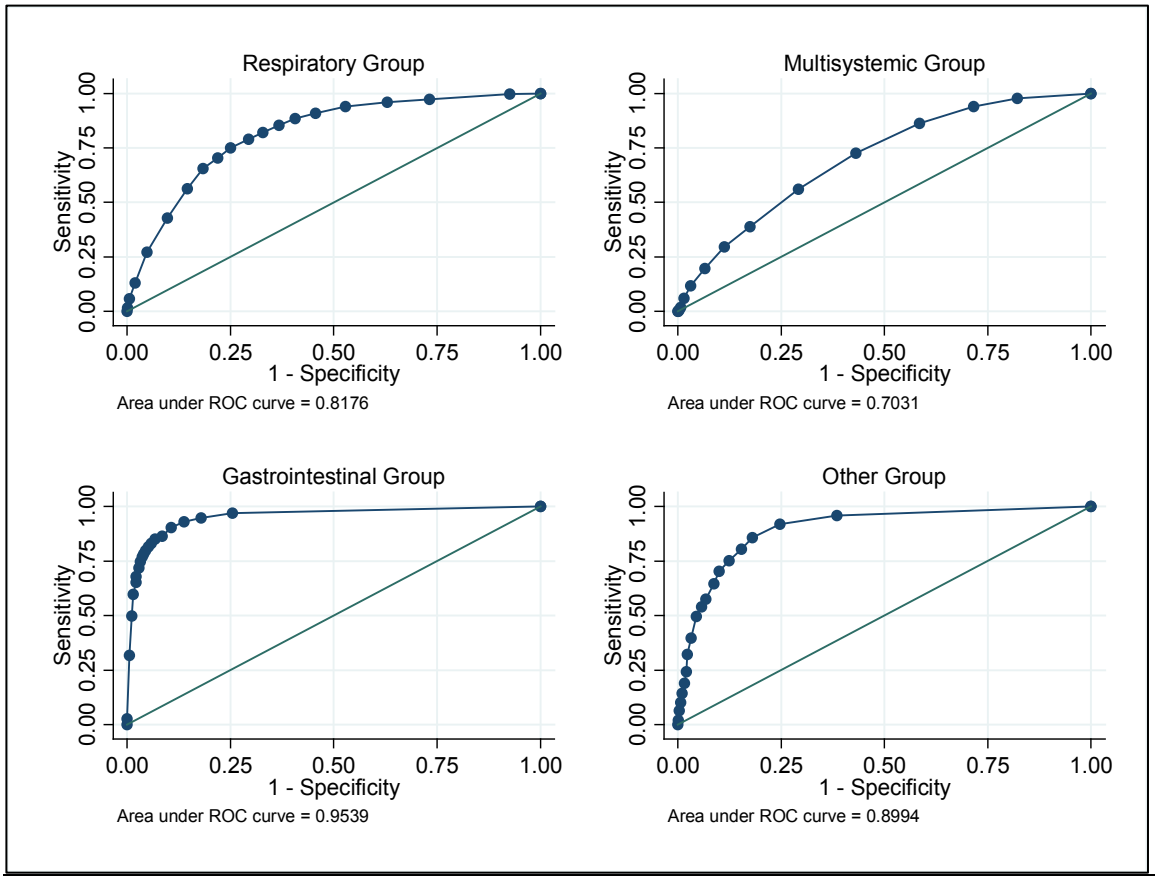
Outliers and influential observations with a multisystemic OS diagnosis: The respiratory and the GI 2 syndromes were the strong predictors in the multinomial model for a Multisystemic OS diagnosis (Table 3). The cross comparisons between all four diagnostic outcomes in Table 5a indicate that PCV syndrome and lymph node submissions were the two that had the most predictive value. Circulatory, neurologic and respiratory had lesser predictive influence. The largest Pearson residuals in the multisystemic logistic model were all positive (> 4). The observations matched to cases with a multisystemic outcome, but had syndromes that were stronger predictors for other diagnoses; respiratory, GI 1, joint and reproductive. One set of high residual observations were GI 1 syndromes with a multisystemic OS diagnosis and linked

to cases from several farms submitted by a single practitioner over a 3 day time period in May 2005. Highly leveraged values occurred in 97 cases with respiratory syndrome, PRRS syndrome and PCV syndrome and a respiratory OS diagnosis. The observations highlight the syndromes that have positive influence on both respiratory and multisystemic OS diagnoses.

Outliers and influential observations with an "Other" OS diagnosis: In table 3, the joint and reproductive syndromes are the two syndromes that predominantly predict "Other" OS diagnosis (negative predictors for all gastrointestinal, respiratory and multisystemic with "Other" as the baseline). High Pearson and deviance residual values were cases with "Other" OS diagnosis, but had two or more of the following syndromes selected; respiratory, GI 1, PRRS or PCV.

The overall sensitivity and specificity of the multinomial model was evaluated at different cut points for each of the OS diagnoses. Figure 3 contains the Receiver Operator Characteristic curves (ROC) and thresholds that optimized sensitivity and specificity. The optimal thresholds were close to the proportions of each OS diagnosis in the full data set. At the optimized thresholds, the sensitivity and specificity for Respiratory diagnosis were approximately equal with a positive predictive value (PPV) of 70.1% and a negative predictive value (NPV) of 79.2%. The area under the curve was greater than 0.8, supporting a good level of predictability. The model also has good sensitivity and specificity for Gastrointestinal and "Other" diagnoses. In these cases, the areas under the curve were close to or above 0.9, supporting excellent predictability. The PPV and NPV for Gastrointestinal was 71.9% and 96.4%. The NPV for "Other" was very similar to gastrointestinal at 96.0%. However, the PPV for "Other" was much lower at 49.0%. The sensitivity and specificity of Multisystemic diagnosis were lowest of all the OS diagnoses in the model. The overall predictability appeared moderate with an area under the curve of 0.7. The NPV was good at 86.1%, but the PPV was very low at 33.3%. The model had a moderate ability to predict non multisystemic cases as non multisystemic, but was relatively poor at accurately predicting true multisystemic cases and had a relatively high false positive rate.

Figure 3: Overall sensitivity and specificity of multinomial logistic regression model with table of ideal thresholds for each Group diagnosis, sample data set.



Group Diagnoses	Threshold	Sensitivity	Specificity	Correctly classified	Ratio
Respiratory	>=0.50	74.96%	74.95%	74.96%	2.99
Multisystemic	>=0.25	55.99%	70.86%	67.80%	1.92
Gastrointestinal	>= 0.25	86.42%	91.58%	90.55%	10.26
"Other"	>=0.20	80.59%	84.65%	84.02%	5.25

To further evaluate the model prediction, the difference in linear predictions was used to compare with the observed OS diagnoses. The difference in linear predictions allowed estimation of whether specific OS diagnosis misclassifications occurred primarily with another diagnosis or were nonspecific (ambiguous). The results in Table 4a and Table 4b were used to compare the difference in linear predictions of the model with either "Other" or Multisystemic set to be the baseline. Misclassifications of Respiratory, Gastrointestinal or "Other" OS outcomes tended to be primarily ambiguous with either baseline. As noted with the sensitivity and specificity, gastrointestinal predictions contained, proportionally, the fewest misclassifications. The linear predictions also supported the high false positive rate for "Other", as the number of ambiguous predictions decreased considerably when the model baseline was changed from "Other" to Multisystemic. Finally the differences in linear

predictions indicated that the model misclassified Multisystemic OS outcomes as respiratory more than any other, including multisystemic and ambiguous. The two outcomes were significantly different in the model, but a misclassified respiratory prediction tended to be multisystemic and the overall ability of the model to predict Multisystemic OS diagnosis was low.

Table 4a: Linear predictions with "Other" as baseline.

	Model Prediction				
OS Outcomes	**Respiratory**	**Multisystemic**	**Gastrointestinal**	**Ambiguous**	**Total**
Respiratory Group	794	44	51	357	1,246
Multisystemic Group	269	79	28	208	584
Gastrointestinal Group	39	4	437	87	567
"Other" Group	68	66	18	286	438
Total	1,170	193	534	938	2,835

Table 4b: Linear predictions with Multisystemic as baseline

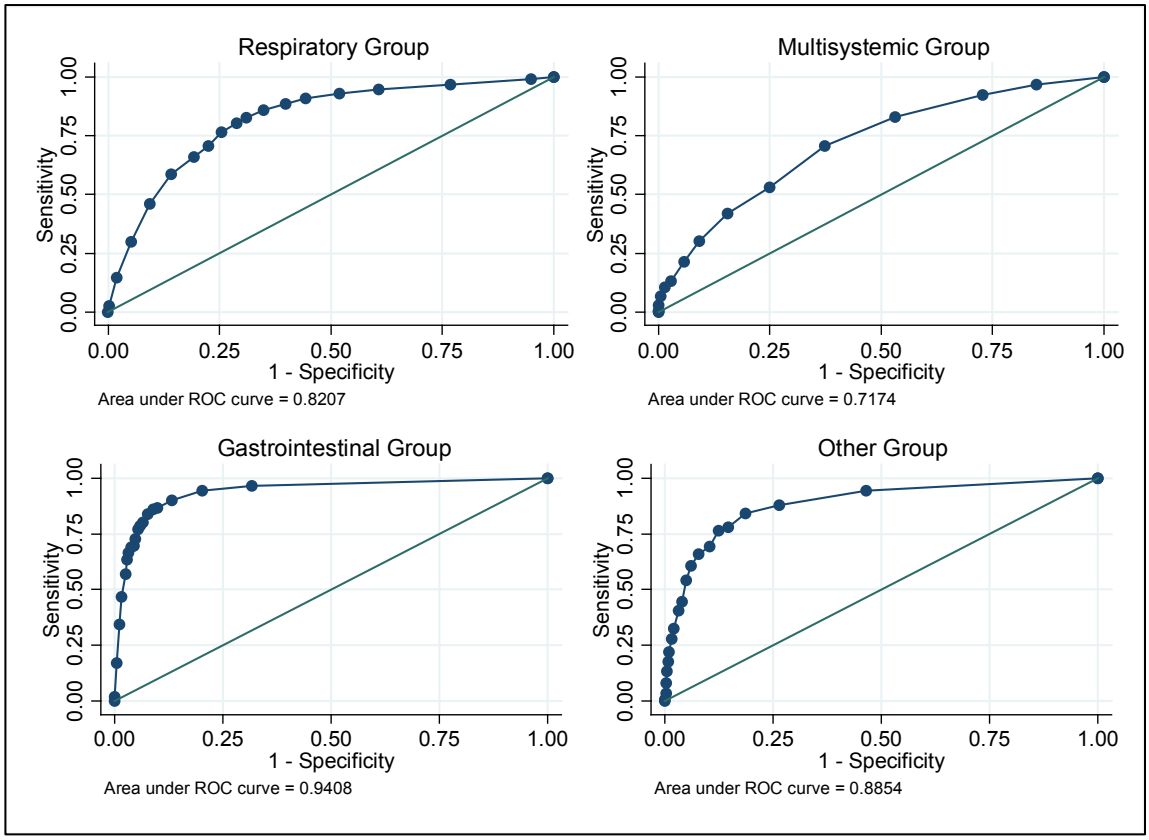
	Model Prediction				
OS Outcomes	**Respiratory**	**Gastrointestinal**	**"Other"**	**Ambiguous**	**Total**
Respiratory Group	888	50	52	256	1,246
Multisystemic Group	363	25	79	117	584
Gastrointestinal Group	46	437	18	66	567
"Other" Group	75	14	239	110	438
Total	1,372	526	388	549	2,835

4.3.4 Syndrome Validation

The overall model predictability was re-assessed for the test data set. Figure 4 contains the ROC curves and thresholds that optimized sensitivity and specificity in the test data set. The optimal threshold, sensitivity, specificity, PPV, NPV and area under the curved for Respiratory diagnosis were very similar to those values for the sample data set. The optimal thresholds for sensitivity and specificity occurred at lower thresholds for the Multisystemic, Gastrointestinal and "Other" diagnoses groups. These thresholds were still close to the actual proportions of each diagnoses group in the full data set. Additionally, the sensitivities, specificities, NPVs and areas under the curve for Gastrointestinal and "Other" were very similar to those from the sample data set. The PPVs dropped slightly to 67.3% for Gastrointestinal and to 46.1% for "Other". The optimal threshold for Multisystemic in the test data supported a higher sensitivity (less false negatives) and a lower specificity (more false positives). However, as

expected the PPV, NPV and the area under the curve changed very little at the lower threshold. The test data reinforced the model's relatively high false positive rate for multisystemic cases.

Figure 4: Overall sensitivity and specificity of multinomial logistic regression model with table of ideal thresholds for each Group diagnosis, test data set.



Group Diagnoses	Threshold	Sensitivity	Specificity	Correctly classified	Ratio
Respiratory	>=0.50	76.67%	74.60%	75.52%	3.02
Multisystemic	>=0.20	70.63%	62.66%	64.25%	1.89
Gastrointestinal	>= 0.20	86.79%	90.07%	89.42%	8.74
"Other"	>=0.15	84.31%	81.32%	81.81%	4.51

The relative risks ratios (RRR) of different syndromes were very similar between the training and test datasets for pairings of OS diagnoses (Table 5a and Table 5b). Five syndromes had RRR that were significantly higher for one OS diagnosis over all the others; PCV (Multisystemic), GI 1, (Gastrointestinal), Joint ("Other"), Reproductive ("Other") and Necropsy ("Other"). The Respiratory syndrome also had a RRR that was significantly higher for the Respiratory OS diagnosis over the other three in the test data, but not significantly different from Multisystemic OS diagnosis in the training data. Two syndromes, Hepatic and Neurologic, were significantly higher for Multisystemic or "Other" OS diagnoses over Gastrointestinal or

Respiratory. The same pattern occurred with the Circulatory syndrome, but only in the test data set.

The marginal effects (ME) of having each syndrome were estimated and the most influential syndromes determined for each OS diagnosis in both the training and test data sets (Table 6). The adjusted predictions due to the most influential syndrome groups were also explored (Table 7).

For the Respiratory OS diagnosis, the average marginal effects in either the test or training data for significant syndromes were within the confidence intervals from the opposite data set (Table 6). The marginal effects of the rhinitis syndrome did not remain significant in the test data. The respiratory syndrome was the best predictor of a respiratory OS diagnosis: Having the respiratory syndrome increased the average probability of a respiratory diagnosis in both the training and test data (0.20 and 0.26 respectively). Alternatively, having a Reproductive, GI 1, Neurologic or Hepatic syndrome decreased the average probability of a respiratory diagnosis. The average adjusted predictions for groups of syndromes from the test data where within the confidence intervals of the training data (Table 7). The exception was the respiratory / rhinitis combination that was not significant in the training data. The adjusted prediction for a Respiratory OS diagnosis was predominantly due to the respiratory syndrome, were cases with the syndrome had average predictions of 0.59 (training data) or 0.60 (test data). Including the rhinitis syndrome, but excluding reproductive and GI 1 syndromes did increase the adjusted predictions considerably (> 0.73). However, the majority of the prediction still came from the respiratory syndrome. The adjusted predictions for respiratory OS outcome for cases that included respiratory syndrome but excluded GI1 and reproductive only increased by 0.035. Rhinitis had a significant marginal effect in the training data (table 6) and impacted the adjusted predictions of syndrome groups in the test data (Table 7). As noted earlier, it also improved overall model fit. However, the rhinitis syndrome was not consistently significant across the test and training data sets. Overall, the respiratory syndrome performed best at predicting a respiratory OS diagnosis. For surveillance purposes, the results indicate that predictions can be further refined for cases that include respiratory and specifically exclude GI 1or reproductive syndromes.

For the Multisystemic OS diagnosis, the average marginal effects of syndromes significant in both data sets were within the confidence intervals from the opposite data set (Table 6). The PCV syndrome had the highest average positive marginal effect across both data sets and was specific to the Multisystemic OS diagnosis. However, the positive marginal effects were lower overall compared to those for the Respiratory OS diagnosis. Several syndromes that had significant marginal effects in one data set were not significant in the other data set. Histology / culture, Respiratory and Lymph node submissions had significant marginal effects in the training data but not in the test data. Likewise, hepatic and reproductive syndromes had significant marginal effects in the test data but not in the training data.

The marginal effects presented in Table 6 indicate that the neurologic, circulatory and hepatic syndromes may have positive marginal effects in the "Other" OS diagnoses or the Multisystemic OS diagnosis: As with the RRR, the neurologic syndrome had a significant positive average marginal effect in the "Other" OS diagnoses and the Multisystemic OS diagnosis, for both data sets. The circulatory syndrome had a positive marginal effect for Multisystemic OS diagnosis in both data sets, but was only significant in the test data set for the "Other" OS diagnoses. Conversely, the hepatic syndrome had a positive marginal effect for the "Other" OS diagnoses in both data sets, but was only significant in the test data for the Multisystemic OS diagnosis. Across the entire analysis, it is important to note that when the average positive marginal effects of a syndrome significantly predicted more than one diagnosis it was always Multisystemic and "Other".

PCV alone had an adjusted prediction of 0.24 and 0.26 for the training and test data respectively. The adjusted predictions of syndrome combinations for Multisystemic OS diagnosis ranged between 0.31 and 0.42 regardless of the data set, with the exception of the PCV/lymph node combination (Table 7). The magnitude of the adjusted predictions depended on syndrome significance within a data set. With lymph node and respiratory syndromes not significant in the test data set, the adjusted prediction of any combination with these syndromes in it dropped compared to those in the training data set. For example, the syndrome combination of PCV with Neurologic, Circulatory, Lymph node and Respiratory had adjusted predictions better than the combination of PCV, Neurologic and Circulatory in the training data, but not so in the test data. The adjusted prediction of Neurologic and Circulatory syndromes increased within either data set by a minimum of 0.053 due to the addition of the

Hepatic syndrome (Table 7). Including the Hepatic syndrome individually with either the Neurologic or Circulatory syndrome had different results. The adjusted prediction of the combination of Neurologic and Hepatic syndromes was very close to the adjusted prediction of the three together in either data set. However, the adjusted prediction of the Circulatory and Hepatic combination was only similar to the three together in the test data set, not the training data set where Hepatic syndrome was not significant for the Multisystemic OS diagnosis (Table 7).

As noted above, neurologic, circulatory and hepatic had varying positive marginal effects on "Other" and Multisystemic OS diagnoses, depending on the test or training data sets. When significant adjusted predictions of combinations of the three syndromes are compared for the "Other" OS diagnoses, it is noted that the combinations that specifically excluded circulatory syndrome or did not involve the circulatory syndrome at all were the only ones with significant adjusted predictions (Table 7). This differed significantly from the adjusted predictions of the combinations with the Multisystemic OS diagnosis.

The GI 1 syndrome was the only syndrome that had a significant positive marginal effect for the gastrointestinal OS diagnosis across both data sets (Table 6). Respiratory, reproductive and joint syndromes had the most significant negative marginal effects. The adjusted predictions were highest when GI 1 was included with respiratory, reproductive and joint syndromes excluded (Table 7). Most of the adjusted prediction was due to inclusion of GI 1 syndrome and the exclusion of the respiratory syndrome. Excluding the other two syndromes only increased the adjusted predictions for training data set by 0.065 and 0.046 for the test data set.

The reproductive and joint syndromes had the greatest positive marginal effects for the "Other" OS diagnoses within both data sets (Table 6). The positive marginal effects of neurologic and hepatic syndromes were described previously. Necropsy syndrome was the final syndrome to have a low positive marginal effect. GI 1 and respiratory had the greatest negative marginal effects. In combination the two syndromes increased the adjusted predictions to 0.692 and 0.765 for the training and the test data set respectively. Excluding syndromes with significant negative influence, the GI 1 and the respiratory syndrome, increased the adjusted predictions to over 0.90 within both data sets. However, it should be noted this would represent a small number of cases.

The results of several other syndromes are also important to note. In particular, Histology / culture did significantly increase the marginal effects of a Multisystemic OS diagnosis and significantly decreased the marginal effects of a Gastrointestinal OS diagnosis in the training data set. However, Histology / culture is not disease specific and it occurs in over 98% of submissions. Additionally, the difference appears to be due to a small number of cases that had a gastrointestinal OS diagnosis without histology or culture. Monitoring this syndrome would likely provide little advantage in early detection over simply monitoring total pathology case submissions. The GI 2 and rhinitis syndromes were not significant in the test data set. The linkages with GI 1 and respiratory respectively could not be validated in spite of how specific these syndromes were. The PRRS syndrome does not significantly affect the marginal effects of any one diagnostic outcome. However, unlike all other syndromes besides PCV, it is a disease specific syndrome for a disease that can occur across all the diagnostic groups. Furthermore it has an overall significant impact on the model. It may be worth utilizing the syndrome to monitor for PRRS.

Overall, the syndrome validation classified the respiratory, GI 1, PCV, reproductive and joint syndromes as strongly significant syndromes linked to specific OS diagnoses. Necropsy also linked to a specific OS diagnosis, but did not have high marginal effects. Neurologic, hepatic and circulatory syndromes linked to two OS diagnoses with the predictive ability for one or the other OS diagnosis improved, depending on the combinations of the syndromes.

Table 5a: Relative Rate Ratios (RRR) of syndromes for pairings of diagnostic outcomes, Training data. Syndrome RRRs > 1 are in bold. Syndrome RRRs with p values < 0.05 or greater included.

	Diagnostic Outcomes Pairs:											
	Respiratory Group			**Multisystemic Group**			**Gastrointestinal Group**			**"Other" Group**		
Syndromes:	MS Grp	GI Grp	Other Grp	Resp Grp	GI Grp	Other Grp	Resp Grp	MS Grp	Other Grp	Resp Grp	MS Grp	GI Grp
Respiratory		**10.66****	**3.73****		**9.37****	**3.28****	0.09**	0.11**	0.35**	0.27**	0.31**	**2.86****
PRRS			**2.22***		**2.15***		0.45*	0.47*	0.40*			**2.50***
PCV	0.52**	**2.05***		**1.93****	**3.96****	**1.58**	0.49*	0.25**	0.40*		0.63	**2.51***
Rhinitis												
GI 1		0.08**			0.11**		**11.82****	**9.39****	**13.71****			0.07**
GI 2			**4.02***			**4.65***			**4.65***	0.25*	0.21*	0.22*
Circulatory		**3.74****			**4.80****	**1.54**	0.27**	0.21**	0.32**		0.65	**3.12****
Hepatic	0.57*		0.43*	**1.76***	**2.63***			0.38*	0.29*	**2.31***		**3.44***
Joint		**17.43****	0.26*		**21.34****	0.32**	0.06**	0.05**	0.02**	**3.78****	**3.09****	**65.94****
Neurologic	0.48**	**2.62**	0.41**	**2.09****	**5.48****		0.38**	0.18**	0.16**	**2.42****		**6.33****
Reproductive	0.29**		0.06**	**3.44****	**11.28***	0.21**		0.09*	0.02**	**16.74****	**4.86****	**54.81****
Histology/Culture		**17.13****			**55.38****	**3.35**	0.06**	0.02**	0.06**		0.30	**16.51****
Necropsy		**1.74**	0.59*		**1.90***	0.64	0.57	0.53*	0.34**	**1.70***	**1.55**	**2.96****
Lymph Node	0.74	**2.90****		**1.35**	**3.91****	**2.05****	0.34**	0.26**	0.52		0.49**	**1.91**

* p>z equals 0.01, **p>z equals 0.001

Table 5b: Relative Rate Ratios (RRR) of syndromes for pairings of diagnostic outcomes, Test data set. Syndrome RRRs > 1 are in bold. Syndrome RRRs with p values < 0.05 or greater included.

	Diagnostic Outcomes Pairs:											
	Respiratory Group			**Multisystemic Group**			**Gastrointestinal Group**			**"Other" Group**		
Syndromes:	MS Grp	GI Grp	Other Grp	Resp Grp	GI Grp	Other Grp	Resp Grp	MS Grp	Other Grp	Resp Grp	MS Grp	GI Grp
Respiratory	**1.97***	**9.39****	**4.47****	0.51*	**4.76****	**2.27****	0.11**	0.21**	0.48*	0.22**	0.44**	**2.10**
PRRS		**2.51***			**2.63***		0.40*	0.38*				
PCV	0.46**	**2.62****		**2.16****	**5.65****	**2.22***	0.38**	0.18**	0.39*		0.45*	**2.55**
Rhinitis			0.45						0.20	**2.24**		**4.89**
GI 1		0.10**			0.14**	**1.94***	**9.75****	**6.99****	**13.53****		0.52*	0.07**
GI 2												
Circulatory	0.65	**3.52****	0.55	**1.54**	**5.42****		0.28**	0.18**	0.16**	**1.83***		**6.43****
Hepatic	0.39**		0.26**	**2.57****	**5.17****			0.19**	0.13**		**3.84****	**7.72****
Joint		**12.85****	0.19**		**16.15****	0.24**	0.08**	0.06**	0.01**	**5.32**	**4.23****	**68.32****
Neurologic	0.56*	**3.42***	0.35**	**1.77***	**6.07****		0.29*	0.16**	0.10**	**2.88**		**9.84****
Reproductive			0.03**		**16.71**	0.06*		0.06	0.00**	**32.29**	**16.78****	**280.40****
Histology/Culture									0.26			**3.84**
Necropsy			0.49*			0.41**			0.31**	**2.05**	**2.46****	**3.18****
Lymph Node		**2.31***			**2.69***		0.43*	0.37*	0.38*			**2.61**

* p>z equals 0.01, **p>z equals 0.001

Table 6: The difference in prediction (marginal effects) of syndromes. All cases are treated as with or without the syndrome, with other syndromes held constant. Predictions with p values ≥ 0.05 listed

		Training Data (2835 cases)			Test Data (1891 cases)		
	Syndrome	M.E.	SE	95% CI	M.E.	SE	95% CI
Respiratory Group	Respiratory	0.198	0.028	0.145, 0.252	0.261	0.034	0.195, 0.327
	Rhinitis	0.214	0.042	0.131, 0.296			
	Reproductive	-0.234	0.040	-0.313, -0.156	-0.257	0.044	-0.341, -0.173
	GI 1	-0.110	0.027	-0.163, -0.057	-0.110	0.031	-0.172, -0.049
	Neurologic	-0.088	0.027	-0.140, -0.035	-0.069	0.034	-0.135, -0.003
	Hepatic	-0.081	0.033	-0.145, -0.016	-0.135	0.040	-0.212, -0.057
Multisystemic Group	Histo/Culture	0.150	0.030	0.092, 0.208			
	Neurologic	0.100	0.026	0.049, 0.151	0.067	0.032	0.003, 0.130
	PCV	0.098	0.021	0.058, 0.139	0.130	0.026	0.079, 0.182
	Lymph node	0.076	0.022	0.033, 0.120			
	Respiratory	0.076	0.022	0.033, 0.119			
	Circulatory	0.064	0.021	0.024, 0.104	0.066	0.025	0.016, 0.116
	Hepatic				0.105	0.041	0.025, 0.186
	Reproductive				-0.076	0.038	-0.150, -0.002
Gastrointestinal Group	GI 1	0.161	0.022	0.117, 0.205	0.169	0.026	0.117, 0.220
	Histo/Culture	-0.225	0.053	-0.329, -0.122			
	Rhinitis	-0.205	0.005	-0.215, -0.196			
	Respiratory	-0.180	0.021	-0.220, -0.139	-0.166	0.024	-0.213, -0.119
	Joint	-0.173	0.016	-0.204, -0.141	-0.168	0.017	-0.202, -0.134
	Reproductive	-0.132	0.036	-0.202, -0.062	-0.176	0.025	-0.224, -0.128
	Circulatory	-0.087	0.017	-0.119, -0.054	-0.102	0.019	-0.141, -0.064
	Neurologic	-0.078	0.020	-0.118, -0.038	-0.101	0.024	-0.148, -0.054
	Lymph node	-0.067	0.017	-0.100, -0.033	-0.062	0.022	-0.106, -0.018
	PCV	-0.059	0.017	-0.091, -0.026	-0.086	0.023	-0.132, -0.040
	PRRS	-0.053	0.018	-0.088, -0.018	-0.064	0.024	-0.110, -0.018
	Hepatic	-0.041	0.018	-0.077, -0.006	-0.080	0.024	-0.127, -0.032
	Necropsy	-0.040	0.013	-0.065, -0.015	-0.036	0.017	-0.069, -0.002
"Other" Group	Reproductive	0.307	0.044	0.220, 0.394	0.509	0.052	0.408, 0.610
	Joint	0.179	0.024	0.133, 0.226	0.231	0.036	0.161, 0.301
	Neurologic	0.066	0.019	0.028, 0.104	0.103	0.028	0.048, 0.159
	Hepatic	0.060	0.024	0.013, 0.106	0.109	0.035	0.041, 0.177
	Necropsy	0.048	0.014	0.019, 0.076	0.075	0.019	0.039, 0.112
	GI 2	-0.096	0.025	-0.145, -0.047			
	Respiratory	-0.095	0.018	-0.130, -0.060	-0.100	0.024	-0.146, -0.053
	GI 1	-0.059	0.017	-0.092, -0.026	-0.079	0.021	-0.121, -0.038
	Lymph node	-0.035	0.017	-0.068, -0.001			
	Circulatory				0.057	0.022	0.015, 0.099

Table 7: The adjusted predictions of the influential syndrome combinations on OS diagnoses, Training and Test data sets

		Predicted probabilities**	
	Syndrome Combinations*	**Training data**	**Test data**
Respiratory Group	Respiratory + Rhinitis - GI 1 - Reproductive	**0.810** (0.747, 0.870)	**0.734** (0.641, 0.828)
	Respiratory + Rhinitis		**0.654** (0.562, 0.746)
	Respiratory - GI 1 - Reproductive	**0.625** (0.598, 0.652)	**0.635** (0.602, 0.668)
Multisystemic Group	PCV + Lymph node + Neurologic + Circulatory + Respiratory	0**.421** (0.355, 0.488)	**0.369** (0.283, 0.455)
	PCV + Neurologic + Circulatory	**0.404** (0.342, 0.466)	**0.373** (0.293, 0.453)
	PCV + Lymph node	**0.281** (0.253, 0.310)	**0.267** (0.232, 0.301)
	Neurologic + Circulatory	**0.348** (0.297, 0.399)	**0.318** (0.250, 0.382)
	Neurologic + Circulatory + Hepatic	**0.401** (0.305, 0.496)	**0.406** (0.291, 0.521)
	Circulatory + Hepatic	**0.329** (0.256, 0.402)	**0.401** (0.310, 0.492)
	Neurologic+ Hepatic	**0.376** (0.286, 0.467)	**0.380** (0.275, 0.486)
Gastrointestinal Group	GI 1 - Respiratory - Reproductive - Joint	**0.761** (0.732, 0.790)	**0.710** (0.673, 0.747)
	GI 1 - Respiratory	**0.696** (0.670, 0.723)	**0.664** (0.629, 0.699)
"Other" Group	Reproductive + Joint - GI 1 - Respiratory	**0.910** (0.864, 0.956)	**0.903** (0.840, 0.967)
	Reproductive + Joint	**0.692** (0.613, 0.775)	**0.765** (0.664, 0.865)
	Neurologic + Hepatic	**0.310** (0.231, 0.389)	**0.311** (0.218, 0.403)
	Neurologic + Hepatic - Circulatory	**0.375** (0.276, 0.475)	**0.476** (0.355, 0.596
	Neurologic - Circulatory	**0.294** (0.242, 0.345)	**0.276** (0.217, 0.335)
	Hepatic - Circulatory	**0.264** (0.212, 0.317)	**0.307** (0.236, 0.378)

***Influential syndrome combinations in model are set as included (+) or excluded (-) to determine adjusted predictions. All other syndromes are held constant (at existing values)**
****95% Confidence Intervals in brackets**

4.4 Discussion

4.4.1 Overview of syndrome classification methods

The syndrome classification method in this chapter included the effective use of a simple analytical approach, agglomerative hierarchical clustering, to group laboratory test requests and specimen types from a regional animal health laboratory (VDS) into syndromes for surveillance. The classification method also included a thorough evaluation of the predictability and validity of the syndrome groups through multinomial regression analysis of training and test data. The intent behind this study was to establish validated syndromes for potential use in the VDS data source regionally and to enhance the contribution of regional swine laboratory data to a national surveillance initiative (Kloeze et al, 2010). Several influencing factors were considered; (a) Syndrome classification focused on the available structured data, as the ability to capture additional data was impacted by limited resources at both VDS and the submitting veterinary clinics. (b) The availability of domain knowledge experts to contribute to rule-based or direct mapping methods was limited, leading to the alternative approaches to syndrome classification. (c) While the data structure was coded, the options of obtaining and modifying a rule-based syndrome classification from another source were not viable as few were available and VDS was not using a standardized disease nomenclature. (d) A simplified method utilizing existing technical capacity, including for automation, was preferred. (e) Finally, a regional multisource approach to early warning surveillance in swine health was considered a long-term goal that would be approached incrementally. The evaluation and analysis of pre-diagnostic indicators from swine pathology submissions for syndromic surveillance was considered a small step in this incremental process.

The syndrome classification method in this study used components that have not been commonly included in animal health syndromic surveillance, such as the hierarchical algorithmic cluster analysis. The challenges, benefits and rational of different syndrome classification methods in animal health contributed to the study approach and are discussed in remainder of this section: Previous syndromic surveillance work has focused on rule-based, direct mapping or supervised learning methods to establish syndrome groups from pre-diagnostic indicators (Dórea et al, 2013; Glickman et al, 2006; Shaffer et al, 2008). One other study was identified as having incorporated hierarchical clustering into syndrome classification, although as part of a multiple factor analysis (Dupuy et al, 2013b). Within animal health

syndromic surveillance systems that have a high degree of data standardization and access to domain expertise, simple rule-based methods have performed well for syndrome classification (Dórea et al, 2013; Hyder et al, 2011; Kosmider et al, 2011). Rule-based methods can also be automated to accommodate the mapping of large numbers of diagnostic or clinical codes into classification schemes (Farkas and Szarvas, 2008; Gibbens et al, 2008). As in public health, these methods are used in systems that are highly structured with standardized nomenclature applied to the data sources involved. Additionally, the systems often have greater access to knowledge experts and are often connected into larger surveillance networks, particularly those in public health (Paiba et al, 2007). From the perspective of early warning surveillance, especially in public health, the use of rule base methods has an advantage in the network approach as the syndrome classifications remain consistent across different data sources and types (Katz et al, 2011; Kloeze et al, 2010). However, the degree of standardization necessary may limit the data sources and types available. In addition, the manual input required to establish and maintain Rule-based methods increases the demand on expert resources and decreases the level of portability and flexibility for application across different data types and structures (Dórea et al, 2013). Both of these limitations are of particular concern for syndromic surveillance based on veterinary medical data.

Supervised machine learning methods, including text mining techniques, have been successfully used to incorporate free text fields from animal health laboratory data into syndrome classification (Dórea et al, 2013). Algorithms that can evaluate the large volumes of free text and semi-structured data components from the multiple data types and/or data sources found in animal health, provide great opportunity to improve syndrome coverage, sensitivity and performance (Kashiouris et al, 2013; Wagner et al, 2004). While not commonly used in animal health, the flexibility of these learning algorithms has been very useful in providing broad syndromic surveillance coverage from multiple data sources and types in public health (Heffernan et al, 2004; Tsui et al, 2003). However, the more common approaches to machine learning methods in syndrome classification utilize input from knowledge experts to establish rules for classification. Additional limitations include a higher degree of complexity for implementation and a decreased transparency for review of the classification process (Chapman et al, 2005a; Dórea et al, 2013).

4.4.2 Syndrome collation through agglomerative hierarchical cluster analysis

The structured format of specimen types and test requests in the VDS LIMS allowed for both to be included in the cluster analysis without having to utilize free text fields or to capture any additional case data for syndrome classification. The example of cluster analysis conducted in this study also allowed syndrome classification on structured data that lacked a standardized nomenclature. Finally, it provided a means, through an unsupervised method, to conduct syndrome classification in situations where resources may limit the availability of domain experts to establish and maintain rule-based methods or establish the case prediction data necessary for supervised learning methods (Hastie et al 2001). The method fit with the existing opportunities and resource implications in the region's animal health surveillance; improving the level of surveillance with existing data while supporting an incremental approach to develop individual components over time with the intent of eventual integration (Hasler et al, 2011; Katz et al, 2011).

An additional advantage of the syndrome classification algorithm in this study is that it provided a simple means to establish multiple syndromes per case for potential identification of complex animal health events. In swine production, as in many animal populations, health events arise from multilevel data where the observation (e.g. laboratory submission) occurs at the animal level, but is representative of and impacted by groups, herds, and regions from which the animals come (Dohoo et al 2009). The result is that disease or other health conditions tend to cluster within multilevel populations, affecting multiple animals in multiple production types (e.g. age groups). Clinical responses in these circumstances lead to evaluation of groups and/or herds with subsequent laboratory case submissions containing representative samples from the multiple animals and production types. As an example, significant swine diseases such as PRRS and PCVAD have a variety of clinical presentations that can differ within the same groups as well as across production types (Cho and Dee, 2006; Madec et al, 2008). The impact on syndromic surveillance with animal health laboratory sources is that cases invariably have multimorbidity, where multiple syndromes may be representative of complex health events. Similar algorithms have been used in the evaluation of complex case management strategies and automated pathogen characterization from high volume data sets (Mi et al, 2012; Newcomer et al, 2011).

The majority of syndrome clusters of test requests and specimens submitted in this study supported the expectations for clinical expressions of swine diseases. The unsupervised

learning algorithms consistently placed respiratory specimens (e.g. lung tissue) and tests for respiratory pathogens (*Mycoplasma hyopneumoniae,* swine influenza) in the same cluster (Pasma and Joseph, 2010; Sibila et al, 2009). Similar clusters were noted for gastrointestinal diseases, reproductive diseases including abortion and musculo-skeletal conditions such as infectious arthritis. Several syndrome groups (Respiratory, Reproductive and GI 1) were consistent with an evaluation of clinic based swine syndromic surveillance in swine (Amezcua et al, 2013). In addition, two algorithm patterns were noted in the cluster analysis; First, the most effective measures of distance between cases were from dissimilarity matrices that weight agreement (what two groups have in common) and ignore differences (e.g. Jaccard). Additionally, the more effective cluster linkages were ones that minimized the variance between cluster groups (e.g. Ward), producing more stable clusters. These patterns are consistent with another study where clustering of clinical conditions identified similar expressions of disease in humans and led to improved management and intervention strategies (Newcomer et al, 2011).

The cluster analysis did not consistently cluster three groups of test requests and/or specimen types; PRRS, PCVAD and lymphatic tissue. Clinical assessment and literature review were utilized to establish these separate syndromes. Unlike non-specific test requests and specimen types (e.g. necropsy, bacterial culture, carcass), test requests for PRRS and PCVAD were targeting specific diseases. The likely reason for the algorithm failure to cluster these groups of test requests is that both diseases have multiple clinical and pathological expressions across different production types, leading to their inclusion with a variety of other test requests and specimen types (Carman et al, 2008; Madec et al, 2008; Young et al, 2010). Similarly, lymphatic tissue is a common specimen type submitted for the diagnostics of a variety of diseases, even though it represents a specific organ system.

A limitation to the methods used in this study is that direct analytical comparisons of classifications from the cluster algorithms relied on post cluster validation of diagnostic outcomes using additional statistical methods, such as regression models. This is because hierarchical cluster algorithms are primarily descriptive and are not amendable to quantitative analysis (Hastie et al 2001). In other machine learning methods, the syndrome cluster algorithms are more amendable to quantitative methods that can assess algorithm

performance through comparisons between the training and test data sets. (Dórea et al, 2013; Hastie et al 2001). For example, more advanced methods can better utilize rules initiated from knowledge experts, allowing the classification algorithms to undergo a more direct, robust comparisons and evaluations through measures such as F-scores. In animal health laboratory data, if reasons for submission, case histories, or diagnostic pathology outcomes are not included in the case level data, then the cluster algorithms used in this study cannot be easily validated. The lack of additional case information limited the data available to pathology cases that had defined outcomes for validation. To use the hierarchical method for syndrome classification of non-pathology submissions, case definitions (for validation) could have been estimated from the numbers of samples submitted per event, herd level disease prevalence and sensitivity/specificity of the tests involved. This approach, while possible, would be highly dependent on ongoing, accurate estimates of prevalence of swine diseases. However, regardless of what cluster algorithim is used for classification of non-pathology submissions, it would be more effective and efficient to capture the minimum data necessary for evaluation in the submission process (Kloeze et al, 2012).

As discussed in Chapter 3, the use of non-pathology submissions for syndrome surveillance may be less valuable to detect emerging or re-emerging swine diseases and more prone to selection bias as the submissions may be for non-diagnostic reasons (Amezcua et al, 2013; O'Sullivan et al, 2012). However, these non-pathology cases represent the bulk of swine submissions to VDS and assumptions regarding their lesser significance may not always apply. Exploring an alternative approach that could be applied to non-pathology submissions would be a long-term consideration.

4.4.3 Syndrome prediction and validation through multinomial regression

The syndrome prediction and validation were effective at estimating the most effective syndromes for use in syndromic methods with the laboratory data. The methods also indicated combinations of syndromes that could be used to increase syndrome sensitivity of complex biological events. The overall predictive ability of the multinomial regression model was moderate to excellent for three of the four organ system diagnoses; Respiratory, Gastrointestinal and “Other”. However, it is important to note that as observed outcomes, the organ system groups are generalized pathology diagnoses for the predictive model and

therefore lack specificity. The lack of "robust" specificity is generally accepted in syndromic surveillance methods because the syndromes precedes diagnostic results with limited additional clinical information (Dórea et al, 2013). The lack of specificity also contributes to inclusion of additional steps that assure cautious interpretation and routine re-evaluation of syndromic methods.

Optimizing the threshold for Respiratory diagnoses to maintain a high specificity would avoid classifying too many false positives, as this would place additional demand on resources for investigations and response. This is counter to approaches taken in hazard-specific surveillance where targeted diagnostic testing maximizes sensitivity in order to avoid missing a significant disease event (Dohoo et al 2009; Hoinville et al, 2013). The predictive syndrome model had limited sensitivity and specificity for multisystemic organ system diagnosis and the model tended to misclassify multisystemic as respiratory. As noted among influential observations, PRRS, PCV, and Respiratory syndromes all had positive influence on both respiratory and multisystemic outcomes. The pathophysiology of several multisystemic swine diseases, such as PRRS and PCVAD, often include a respiratory component or secondary infections with respiratory pathogens (Madec et al, 2008; Young et al, 2010; Zimmerman et al 2012). Differential diagnoses may include respiratory diseases such as swine influenza and *M. hyopneumoniea*, leading to respiratory testing and a predictive model outcome of respiratory. This explanation is consistent with the multiple correspondence analysis (MCA) results (Chapter 3) where a primary multisystemic diagnosis frequently had additional respiratory diagnoses, leading to low dimensional variation between primary respiratory and multisystemic diagnoses. The low dimensional variation likely contributed to inclusions of primary multisystemic diagnoses in the respiratory organ system group used for validation. The significant positive impact of increasing numbers of test requests on the likelihood of predicting a respiratory OS outcome also supports the explanation, as the number of test requests for such complex cases would be higher.

The syndrome validation using a test data set and comparison of adjusted predictions was very effective in identifying the significant syndromes developed through the unsupervised learning algorithms. Adjusted predictions allowed the evaluation of a syndrome or combination of syndromes while holding all other syndromes constant for the outcome of interest (Stata Press 2009). The respiratory syndrome represented test requests for very significant swine disease

where early warning surveillance would be useful. In particular the syndrome included swine and pandemic influenzas that have zoonotic disease implications (Pasma and Joseph, 2010). The model prediction did not indicate a significant difference in the relative risk for the respiratory syndrome between the respiratory and multisystemic diagnoses in the training dataset. However, the adjusted predictions within the test and training data sets indicated that the respiratory syndrome had a considerably better prediction for a respiratory diagnosis. Similarly, the necropsy syndrome was generally a non-specific syndrome that indicated only whether a pathologist or clinical veterinarian conducted the initial necropsy. The relative risk of an "Other" diagnosis for necropsy was significantly different than for the other three diagnoses. However, the adjusted predictions for the necropsy syndrome were quite low, suggesting that most of its impact was related to inclusion with other more disease specific syndromes. Finally, adjusted predictions of syndrome combinations such neurologic, circulatory and hepatic possibly provide a sensitive indicator for diseases like neonatal septicemia (neurologic + circulatory) and may also indicate that clustering the combinations into new syndromes is warranted.

An important additional consideration in syndrome classification and validation is the sensitivity for unknown or rare events, such as bioterrorism events, foreign animal diseases or emerging diseases. Evaluation and analysis of the validation data should include surrogate or grouped outcomes that are representative of these events as the events themselves are usually not found in available data (Gibbens et al, 2008; Katz et al, 2011). In this study, several key validated syndromes have to potential to identify rare events. The neurologic and respiratory syndromes would be important to identify foreign diseases such as pseudorabies. The neurologic syndrome in particular not only includes neurologic tissue, but also testing for differential diagnosis such as neonatal septicemia (e.g. *Streptococcus suis*) (Zimmerman et al 2012). Similarly, the acute form of another foreign disease, classical swine fever, exhibits circulatory signs that may be detected through the circulatory syndrome (Zimmerman et al 2012).

Another important consideration is the impact of external factors on syndrome sensitivity and performance. Seasonality of disease, changes in population demographics, incursion of an emerging disease and economic factors may influence both the level of activity providing the

surveillance data as well as the actual prevalence of diseases and in turn impact the accuracy of the syndrome groups (Burr et al, 2006). The implications for this study can be noted with the comparative significance of the second and third 25-month time periods for multisystemic diagnosis (over "Other" and the first time period). These time periods coincided with an increase in prevalence of PCVAD in the Canadian and Manitoba swine populations (Carman et al, 2008; Madec et al, 2008; Poljak et al, 2010). The prevalence of PCVAD has since declined due to improved biosecurity standards and an effective vaccine (Verdon et al. 2012). The impact of an increasing disease prevalence within the data may have impacted the cluster algorithms and subsequent validation of some syndromes, most notably, the PCV syndrome. As discussed earlier, a limitation to unsupervised learning algorithms is the necessity to conduct full validation to effectively evaluate the syndrome clusters heuristic means. The syndrome classification and validation would need to be repeated retrospectively on a current data set in order to assess the significance of the time periods on syndrome prediction.

Automation of the syndrome classification within the VDS LIMS was not fully evaluated in this study. The predictive modelling and validation used in this study identified the most effective syndromes within the historical data. An automated process could be used to map cases to the key syndromes identified based on the same methods applied above. Using the internal coding within the LIMS, a case would be assigned a syndrome if one or more test requests and one or more specimen types (if present) within the syndrome were recorded in LIMS from the case submission. In order to map cases, a key step in automation would be to reduce the data to the case level and assign the syndromes as variables. An additional, complex automation component would evaluate cases with specific combinations of syndromes. From the marginal effects for both the training and test models recorded in table 7, an automated process would flag cases that contain multiple syndromes, such as respiratory and rhinitis syndromes, PCVAD and lymph node syndromes, or neurologic, hepatic and circulatory syndromes. The combinations could be used to refine syndrome sensitivity or to target specific conditions.

Two options have been explored for the technical implementation of automated syndrome mapping. One option would utilize a formatted data set, with individual identifiers removed, that is automatically extracted from the LIMS on a daily basis for transfer to a national animal health surveillance network (Kloeze et al, 2010). Procedures have been developed to copy the

extract to a secure server for further analysis, but have not been implemented. The data management and analytical components for syndrome mapping have been developed using a statistical software package (StataCorp, 2009). The statistical software is capable of automation, but it is likely the mapping would need to be initiated manually. As a result this option would be resource intensive and limit the timeliness of the syndrome mapping. Since a key feature of syndromic surveillance methods is real time or near real time functionality, further work would be need for this option to be utilized (Hoinville et al, 2013; Katz et al, 2011). The second option was explored as part of the original intent of the study; the utilization of query tools developed in the national animal health surveillance network to detect meaningful events in multiple data sources. The network query tools are contained in a Dynamic Syndrome Builder™ (DSB) that allows users to develop syndromes from accessible data on the network. The methods are rule-based and require the user to know either key terminology or individual codes in the data sources for syndrome mapping. The DSB™ tools allow data to be reduced to the case level and allow sets of syndrome maps to be saved and rerun through a single step at any time. Additionally, the tools are complex enough to incorporate inclusionary/exclusionary components to the syndrome mapping and allow implementation of syndrome combinations. However, the component is not automated and manual extraction of the data would be required for further detailed analysis. The DSB™ tools have been used periodically with VDS data to establish targeted, disease specific syndromes for ongoing monitoring (e.g. *Salmonella* Enteritidis in poultry, Influenza in swine).

4.4.4 Conclusion

The syndrome classification approach used in this study effectively grouped syndromes from the test requests and specimen types submitted to an animal health laboratory for use in syndromic surveillance in a regional swine population. The algorithms demonstrated a repeatable and readily adaptable process to cluster structured data that contained only the basic submission information and lacked standardized disease nomenclature. While a level of expertise was required in order to ensure the clinical relevance of the syndromes, the approach also decreased the domain knowledge requirements for syndrome classification. The approach was able to accommodate multiple syndromes per case, which was considered more representative of the complex multilevel nature of swine populations. Finally, the use of cases with pathology diagnosis allowed for an effective and robust syndrome validation using a

predictive model. The validation step allowed for the generation of sensitivities and positive predictive values for syndromic methods, as well as estimations of adjusted predictions syndromes, individually or in combination. The syndrome classification and validation will need to be manually revised and repeated on a periodic basis to ensure syndrome sensitivity and performance. The methods use standard statistical software that can utilize the data coding structure and maintain analytical models for subsequent analysis.

The limitations to the data and methods in this study include the necessity of a robust validation using defined case outcomes, as hierarchical cluster analysis does not provide an analytical means of syndrome prediction or comparison. In the laboratory data used within this study, cases with pathology diagnoses were considered the most relevant for early warning surveillance and therefore could be used for further validation. However, pre-diagnostic data in non-pathology cases, which made up the majority of submissions, could not be included in the syndrome classification algorithms because case definitions were not easily achievable for validation. The inclusion of a recommended minimum data set within the data source would prospectively improve the use of non-pathology submissions, especially data fields that would capture disease classification by submitter (Kloeze et al, 2012).

Syndrome mapping and automation are the next steps in the development of the syndromic components. Two options are available for further exploration, refinement and implementation, including one that provides linkage to national animal health surveillance initiatives. However, further work is required in both options to fully automate the mapping of syndromes in real time.

The results of this study demonstrated a reliable and robust means of classifying and validating a syndrome set for the application of syndromic surveillance methods in a regional animal health laboratory. The methods are incremental steps in developing the components of an early warning surveillance system for the detection of significant animal health events in a regional swine population.

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Chapter 5: Thesis summary and future considerations

Each chapter of the thesis has included findings, considerations and implications of the evaluations and analyses conducted. The purpose of this chapter is to focus on several key areas for review and for further evaluation, analysis and/or implementation. These will include: (a) The fit of animal health syndromic surveillance within a One Health framework. (b) Evaluation of key factors that impact swine submissions to Veterinary Diagnostic Services (VDS) and potential submission improvements to meet a minimum data set for surveillance. (c) Alternative methods for syndromes and for syndrome classification, including the use of disease codes and the potential for other unsupervised and supervised classification algorithms. (d) Further work in the areas of temporal analyses and aberration detection, including the development of baseline data. (e) Finally, a review of the process steps and potential integration methods that would contribute to the incremental development of an early warning animal health surveillance system for a regional swine populations.

5.1 The fit of syndromic surveillance methods within a One Health framework

As stated in chapter 1, effective animal health surveillance is an essential part of evidence based decision making required to protect animal and public health, to provide assurance of a healthy food supply, to support economical and sustainable livestock production and to protect the intrinsic value of animals for the public good (Hasler et al, 2011; Hyder et al, 2011; Lysons et al, 2007). Effective animal health surveillance is an integral part of multilevel (regional, national and global) approaches to managing the increasing health threats to humans, livestock, pets and wildlife from endemic, emerging, re-emerging and exotic diseases (Dupuy et al, 2013a; Hasler et al, 2011; Zinsstag et al, 2011). The health threats to people, animals and the environment have arisen from complex global factors including extensive patterns of climate change, the rise in human and animal populations, greater interconnections between humans and animals, as well as the high degree of transboundary trade and travel (Dupuy et al, 2013a; Wendt et al, 2014; Zinsstag et al, 2011). Effective multilevel approaches to these threats are moving towards a cross-sector integration and collaboration in surveillance, mitigation and intervention as part of a “One Health” framework (Wendt et al, 2014; Zinsstag et al, 2011). In the context of “One Health”, the challenges for animal health surveillance are to effectively provide the descriptive information and detailed analysis of animal health hazards necessary for risk mitigation and intervention from a broad spectrum of information sources,

types and purposes. Traditional hazard specific surveillance methods and disease mitigation programs continue to be necessary to target specific diseases in at risk populations, but are often too focused and resource demanding to address multiple unpredictable threats (Dupuy et al, 2013a; Hoinville et al, 2013; Vrbova et al, 2010). Using methods such as syndromic surveillance, early warning disease surveillance systems have been adapted to detect unusual or unexpected events in health related behaviours from a broad range of data types and sources (Dorea et al, 2011; Dupuy et al, 2013a; Hoinville et al, 2013; Katz et al, 2011; Zinsstag et al, 2011).

In the context of public health specifically, animal health data may contribute in two ways; (1) As a direct source for human health surveillance, such as detection of zoonotic pathogens in animal populations that are usually asymptomatic in animals (e.g. *Salmonella* Enteritidis in poultry, *E. coli* O157:H7 in cattle) (Nesbitt et al, 2012). (2) As a indirect source where systems detect significant zoonotic diseases in animals that may be a risk for human health, such as in sentinel surveillance (e.g. Rabies programs in wildlife and domestic pets, parasite monitoring programs) (Blanton et al, 2008). In the greater context of One Health, animal health surveillance also functions to detect diseases that may have a significant economic impact, such as foreign animal diseases; or that may impact the environment, such as antimicrobial resistance in environmental organisms (Busani et al, 2006; Gibbens et al, 2008). To assess the efficacy, coverage, validity and opportunities for integration of animal health surveillance within the "One Health" framework, research and investigation into surveillance methods and systems have been identified as important actions, especially as methods such as syndromic surveillance become more frequent in development and use (Dorea et al, 2011; Dupuy et al, 2013a). The preceding chapters provide examples of addressing some of these steps.

5.2 Improvements to an animal health laboratory data source for syndromic surveillance

The evaluation of the Laboratory Information Management System (LIMS) within Veterinary Diagnostic Services (VDS) in Manitoba for the purposes of syndromic surveillance was consistent with evaluations of other animal health laboratories (Dorea et al, 2013; Shaffer et al, 2008). In particular, the accessibility, centralization, electronic data capture and internal structure were comparable. However, a key assumption that warrants further exploration is the use of submission information (test requests and specimen types) as effective, representative indicators of health related behaviours in the populations of concern. The

assumption centres on the increased likelihood of practicing veterinarians submitting samples to a diagnostic laboratory when encountering emerging diseases, changes in endemic diseases, diseases that have sudden and severe clinical impacts, or have significant human health risks (Dorea et al, 2011). It is recognized that submission bias through the role of the practicing veterinarian may affect the usefulness of the data for surveillance purposes. Submission bias is impacted by the experience of the submitting practitioner, convenience of laboratory submission, timeliness of laboratory diagnoses, relationship of veterinarian to producer (consultant, employee), overall herd management and current industry economics (Bartlett et al, 2010; O'Sullivan et al, 2012; Sawford et al, 2013). An analysis of economic and disease factors affecting swine laboratory submissions in Ontario and a focused ethnographic study of Alberta cattle veterinarians have reported key factors that encouraged diagnostic laboratory submissions (O'Sullivan et al, 2012; Sawford et al, 2013). These factors recognize that disease outbreaks are a significant reason for laboratory submissions. Sawford, through qualitative means, reported that cattle veterinarians who encountered cases where a clinical diagnoses was not reached, where a reportable disease was expected, were considered atypical or bizarre, or were considered significant public health risks, were more likely to submit samples to diagnostic laboratories (Sawford et al, 2013). Similar methods could be utilized to determine impacts on laboratory submission from Manitoba swine practitioners.

A combination of improved submission information and improved data structure would assist in the use of VDS data for syndromic surveillance. The addition of key submission information would provide the opportunity to improve surveillance performance and syndrome classification. Based on a recommended minimum data set for the collation and analysis of animal health laboratory data for surveillance, several key fields in the VDS LIMS were missing, inconsistent or collected in a manner that made the information not readily accessible (Kloeze et al, 2012). One example is the use of a farm identifier within the VDS LIMS to link an animal health event to a specific livestock premises. A premises identifier provides the information necessary to ensure multiple health events are occurring at one location (or herd) or at multiple locations, an important factor in determining if related animal health events are impacting groups of animals on multiple locations (Kloeze et al, 2012). In the VDS LIMS, these location identifiers were assigned by laboratory staff based on submission information. In the data used in this study, identification errors could have occurred over time if individual farms

were assigned different identifiers from inconsistent submission information provided by veterinarians. The recent implementation of a Premises Identification Database (PID) for all livestock and poultry operations in Manitoba allows for the use of a coding system based on legal land descriptions as a standardized means of identifying individual farms within the LIMS while maintaining confidentiality.

Another example would be the inclusion of "disease classification by submitter" as additional submission information to refine syndrome classification. The inclusion of clinical diagnoses to improve syndrome classification have been implemented using incentivized, practitioner derived disease category in other animal health early warning surveillance systems (Berezowski et al, 2011b; Gibbens et al, 2008; Vourc'h et al, 2006). The inclusion of terminology within the classification list for unknown or unidentified conditions, such as "diagnosis not reached" has been demonstrated to improve the surveillance for emerging diseases (Hyder et al, 2011; Kosmider et al, 2011). Furthermore, coded or free text practitioner submission information can be mapped to a standardized disease nomenclature similar to syndrome classification in public health syndromic surveillance (Betancourt et al, 2007; Chapman et al, 2005; Conway et al, 2013). Currently, VDS submissions frequently include free text clinical descriptions provided by veterinarians as part of case histories. However, in common with the situation described for other animal health laboratory data, the information provided was not available in the LIMS (Dorea et al, 2013). If the free text case histories were to be included in the LIMS, the use of free text syndrome classification techniques, such as supervised learning algorithms (e.g. Naive Bayes), may provide improved syndrome classification (Conway et al, 2013; Dorea et al, 2013; Farkas and Szarvas, 2008). However, it may be more practical and efficient to structure submission information into coded diagnostic categories that could be classified for syndromic surveillance using Rule-based methods (Dorea et al, 2013).

5.3 Alternative approaches to syndrome and outcome clustering

Unsupervised clustering methods are used to detect natural clusters in the data without predictive information to "train" the algorithms beforehand (Hastie et al. 2009). Unsupervised methods have not been commonly used for syndrome classification of pre-diagnostic indicators. Association rules, K means and agglomerative hierarchical clustering are relatively common unsupervised methods used to classify outcomes in health fields such as diagnostic

imaging and genetic mapping of pathogens (Hastie et al 2009; Mi et al, 2012; Zou et al, 2013). For syndromic surveillance these methods are useful in circumstances where the pre-diagnostic indicators are less directly linked to diagnostic outcomes, as is the case with indicators from pharmacy sales or abattoir condemnations (Dupuy et al, 2013b; Wallstrom and Hogan, 2007). Without additional case history and clinical diagnoses for syndrome development, laboratory test requests and specimen types can be considered less directly linked to diagnostic outcomes when used alone for syndrome classification. Unsupervised methods may also be useful when case definitions for syndrome mapping are impacted by a lack of standardized disease nomenclature and/or by limited resources for routine application of knowledge matter expertise (Chapman et al, 2005; Dorea et al, 2013). The disadvantages of unsupervised learning methods include: (a) less transparency and interpretability than rule-based methods which can impact acceptance (Dorea et al, 2013); (b) without the comparative training/test methods used in supervised learning, the cluster patterns require post algorithm validation through statistical methods (Hastie et al 2009). Given that pathology outcome data may often be available for syndrome validation, further evaluation and comparison of unsupervised (e.g. k means, association rules) and supervised (e.g. naive Bayes classifiers) methods could be conducted retrospectively with the VDS data to determine a preferred means for syndrome classification. Additionally, a comparison of unsupervised and supervised methods may provide an opportunity to develop more meaningful syndrome classifications for non-pathology diagnoses.

Outcome clusters for syndrome validation from pathology submissions could also be developed from the disease diagnoses rather than the organ system diagnoses. This approach may be reasonable as the evaluation conducted in Chapter 3 demonstrated that the 30 most common primary diagnoses codes accounted for just under 50% of all pathology cases assigned to organ systems in the data set (4 from Multisystemic, 7 from Respiratory, 9 from Gastrointestinal, 10 from “Other”). As with organ systems, clustering methods would have to account for disease codes used in additional diagnoses (i.e. secondary, tertiary, etc), but should offer the opportunity to develop more specific outcome groups. The use of disease diagnoses would complicate the analysis of pathology outcomes by increasing the number of possible outcomes. However, other studies have utilized similar methods to address large numbers of interrelated outcome observations. For example, a three step procedure using Multiple correspondence

analysis (MCA), hierarchical clustering and a k means algorithm have been used to cluster abattoir condemnation and wildlife necropsy data into “syndromes” for surveillance purposes (Dupuy et al, 2013b; Warns-Petit et al, 2010).

The use of disease instead of organ system diagnoses offers an alternative option for early warning surveillance: Diagnostic outcome clusters could be used as the primary indicators for a laboratory based early warning surveillance method. Compared to traditional “passive” methods of monitoring pathology cases, clustering diagnostic outcomes into relevant groups would allow for temporal analysis and aberration detection, avoiding excess signal “noise”. Compared to pre-diagnostic indicators, diagnostic outcomes clusters represent the final diagnoses for individual cases, leading to greater surveillance sensitivity and specificity relative to real health events. The approach would be similar to other syndromic surveillance methods that used “post diagnostic” data, such as abattoir condemnations and wildlife necropsies (Dupuy et al, 2013b; Warns-Petit et al, 2010). The approach has a further advantage of capturing “No specific diagnoses” as an indicator of unknown or unexpected events. As described in Chapter 3, “No specific diagnoses” was a disease code that exhibited an increased trend in pathology cases during the initial incursion of Porcine Circovirus Associated Disease (PCVAD) into the Manitoba swine herd. A similar coding (“Diagnosis Not Reached”) was successfully used in the United Kingdom’s Veterinary Investigation Diagnosis Analysis system (VIDA) to identify emerging disease issues (Hyder et al, 2011; Kosmider et al, 2011). The key disadvantage is the loss of timeliness, as pathologically comfirmation associated with cases may take from several days to a few weeks to be completed. The approach would not meet the definition of syndromic surveillance as it would not use available pre-diagnostic indicators and the timeliness would not be “real” time (Hoinville et al, 2013). However, the approach could still provide an enhancement of the traditional animal health laboratory roles in surveillance and may provide early warning of diseases that are slower to manifest, such as some emerging diseases (e.g. PCVAD) or to changes in endemic diseases (O'Toole, 2010).

5.4 Temporal analysis and aberration detection

Syndromic surveillance relies both on effective syndrome classification and on the timely detection of significant aberrations in syndrome clusters to indicate real disease events (Dorea et al, 2012; Hoinville et al, 2013). The purpose of temporal analysis and aberration detection is

to compare the current case count for a given syndrome with a threshold derived from historical data, using analytical means to detect significant disease anomalies (Kosmider et al, 2011). To ensure that outbreak signals in prospective surveillance are not obscured or falsely elevated, historical baselines must account for cyclical temporal factors (e.g. day of week, season), global trends (e.g. previous outbreaks, economic factors) and/or autocorrelation (e.g. repeated herd testing over time) in submission patterns. In animal health syndromic surveillance, baselines can be established using retrospective time series analyses based on regression models that account for trends in the data (Dorea et al, 2012; Hohle et al, 2009). Poison regression models have been found effective if the assumptions of equal variance and mean are met. However, in many circumstances the Poison assumptions are not realistic, with the additional complication of significant time periods having zero counts. Negative binomial models, with or without a zero inflation parameter, can provide effective alternatives in these situations (Hohle et al, 2009; Thomas-Bachli et al, 2012). Alternative univariate methods have utilized a log linear regression approach to account for baseline trends and for prospective aberration detection (Hohle et al, 2009; Kosmider et al, 2011). However, this method assumes a constant submission rate over a given time period and address seasonal trends through weighted averages or percentiles, and does not specifically address global trends.

In order to effectively establish a temporal baseline for prospective surveillance, the retrospective data must be free of outbreaks (Dorea et al, 2012). The presence of previous outbreaks in the baseline will influence the threshold for a syndrome such that the sensitivity for significant aberrations will likely be reduced. Unfortunately, the syndrome validation conducted in this study (Chapter 4) identified a significant impact of Multisystemic Organ system diagnoses over three consecutive 25-month time periods. The time periods were ordinal indicators of anomalies in submission trends and were potentially representative of the incursion of PCVAD into the Manitoba swine herd in 2005. Prospective surveillance with syndromes that predicted Multisystemic organ system diagnoses (e.g. PCV) could lack sensitivity if a historic baseline were established from the data set. Since the influence was significant within three 25-month periods, non parametric techniques used by others, (e.g. moving averages, moving percentiles) may not be sufficient to smooth the aberration (Dorea et al, 2012; Kosmider et al, 2011). To account for aberrations in baseline data, Dorea utilized a Poisson regression model technique that detected and replaced outliers above the 95th

percentile of the Poisson distribution with model predicted values (Dorea et al, 2012). This approach was able to address a global trend in the laboratory data related to a single year increase in bovine leucosis virus (BLV) testing. A similar approach may be necessary to prepare a temporal baseline for syndromes that predict Multisystemic organ system outcomes from the VDS LIMS.

The primary goal of any syndromic surveillance method is to detect accurate signals of significant disease events in continuously monitored data. The process of signal detection from prospective monitoring of syndrome counts within the VDS LIMS requires automation of both syndrome assignment and signal detection. Two options for automated syndrome assignment were discussed in Chapter 4. Either option would require additional programming for the application of automated signal detection algorithms. Notwithstanding the current software limitations, there have been several methods used in animal and public health for effective outbreak detection from temporal and spatial signals. Perhaps the most common approaches to outbreak detection are statistical process control (SPC) methods, such as CUSUM, where algorithms evaluate cumulative sums over moving baseline time periods to detect if a change point (threshold) has been reached (Hohle et al, 2009; Shaffer et al, 2008; Tokars et al, 2009). The change point is derived from an analytical process (e.g. Likelihood ratios). The methods do not perform as well in situations with significant daily variation or with low syndrome counts, but are easier to implement and can accommodate season trends. Furthermore, the simplicity compared to other methods, their frequency of use and the different algorithms available make SPC methods appealing for outbreak detection in syndromic methods applied to the VDS data

Spatiotemporal scan statistics are also common for early warning surveillance in animal health when spatial data were available (Hyder et al, 2011; Odoi et al, 2009; Thomas-Bachli et al, 2012). These methods detect localized excess of events where a likelihood ratio is used to compare the number of cases within windows (in both space and time) to an expected number based on the cases surrounding the windows (Kulldorff et al, 2007). An iterative maximum likelihood approach is used to determine the most likely cluster from multiple scanning windows. Regression models can be included to establish baselines through multivariate retrospective analysis (Hyder et al, 2011; Thomas-Bachli et al, 2012). With the inclusion of

premises identification in the VDS data, spatiotemporal analysis could be considered an option for outbreak detection in the syndromic methods.

Additional outbreak detection methods used in syndromic surveillance include time series methods, such as Autoregressive Integrated Moving Averages (ARIMA), and Generalized Linear Models (GLM). Adaptations of these methods for prospective outbreak detection have been used in public health syndromic surveillance (Murphy and Burkom, 2008; Reis and Mandl, 2003). While common in retrospective analysis, the methods are not commonly used for outbreak detection in syndromic surveillance for animal health.

5.5 Review of processes required for implementation and potential for integration

This chapter has built on the evaluation and analyses conducted in the previous chapters and explored several key concepts and methods that are applicable to syndromic surveillance for the Manitoba swine population, utilizing data from a regional animal health laboratory. The further development of the research within these chapters will require strategic, incremental application of core concepts and processes with recognition of limited resources and time. A comprehensive approach will continue to include a "One Health" framework to provide opportunities to address significant disease risks and health events across people, animals and the environment. The approach should allow for integration and collaboration to address multiple disease risks for swine and humans, such as influenza, enteric infections (e.g. *Salmonella*) and antimicrobial resistance. It should also strive to establish shared public and animal surveillance platforms that can address swine specific diseases, such as PCVAD and Porcine Epidemic Diarrhea (PED). Examples of such shared approaches include the Canadian Animal Health Surveillance Network (CAHSN) and the Canadian Science Center for Human and Animal Health. The latter led the development of diagnostic tools for PED during the 2014 Canadian outbreak. Building on the close linkages between VDS and organizations such as CAHSN will continue to be an important process.

The process to implement laboratory based syndromic surveillance for swine should include several important next steps. First, a review of the syndrome classification and validation methods would ensure that syndromes reflect the current laboratory data, especially the currently available test requests and specimen types. K means clustering could also be explored as an additional unsupervised syndrome classification method as the pre processing

method (i.e. dissimilarity matrices) applies to both k means and hierarchical clustering. The second step would involve an exploration of methods for automation through the CAHSN system. Utilizing CAHSN would provide a more timely strategy than applying automation methods directly within the VDS LIMS. The strategy would also allow for syndrome system development that would benefit both VDS and CAHSN. The final steps would involve establishing a baseline for syndrome cluster detection and implementing a reliable outbreak detection method. These final steps would be reliant on the successful implementation of the preceding steps and available resources.

As a final consideration, the implementation of early warning surveillance in public health has benefited from the integration of multiple data sources and types (Katz et al, 2011; Nesbitt et al, 2012; Wendt et al, 2014). Similar approaches have occurred globally in animal health surveillance where integration has occurred across multiple animal health data sources and/or with public health systems (Dupuy et al, 2013a; Lysons et al, 2007; Paiba et al, 2007). In Canada, the province of Alberta has established a broad based surveillance, intervention and mitigation system, which started with clinic-based data sources for cattle. The system has developed signal detection methods, incorporated laboratory and abattoir data, and established a disease investigation response to address any significant animal health events identified (Berezowski et al, 2011a). Research and analysis into early warning surveillance has also progressed in Ontario. In swine, the early warning surveillance methods have included practitioner-based and abattoir condemnation data (Amezcua et al, 2013; Thomas-Bachli et al, 2012). In cattle, the methods evaluated have included laboratory submission and abattoir condemnation data (Alton et al, 2012; Dorea et al, 2012; Dorea et al, 2013). In the case of the research outline above, the high degree of specialization within Manitoba swine practitioners and the availability of data from a provincial meat inspection system, provide opportunities to explore an early warning surveillance initiative for swine beyond the laboratory methods.

In summary, the evaluation and analysis presented in these chapters support the application of syndromic surveillance methods to pre-diagnostic data from an animal health laboratory for the purpose of early warning surveillance in a regional swine population. The methods and the source discussed are well suited to contributing important components in the incremental

process of developing a regional swine surveillance system, utilizing multiple sources and methods, situated within a One Health framework.

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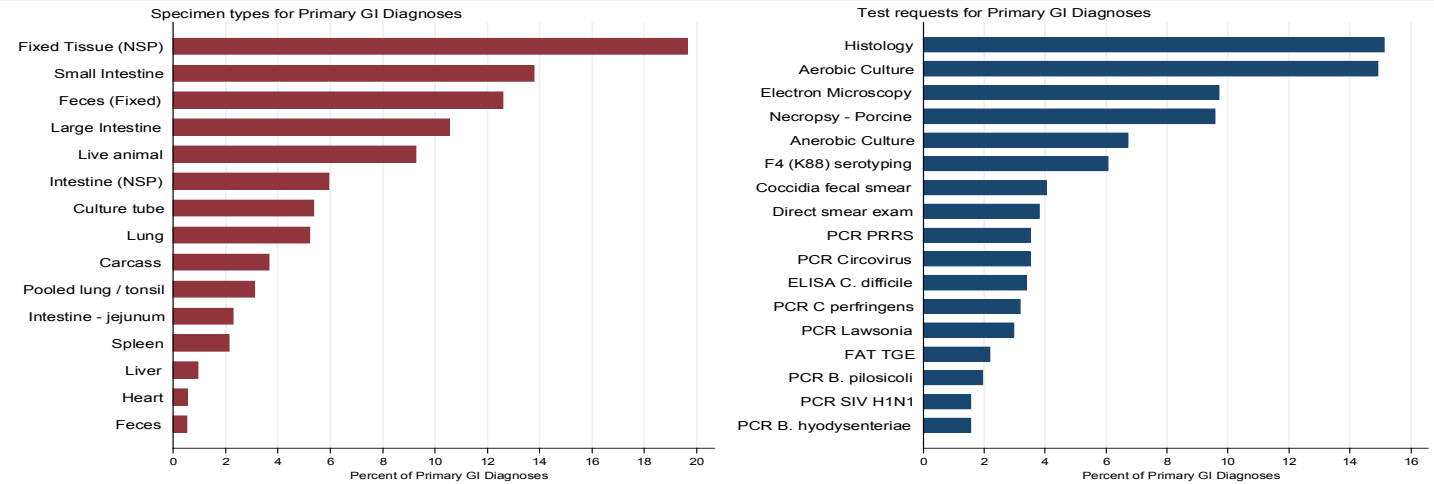
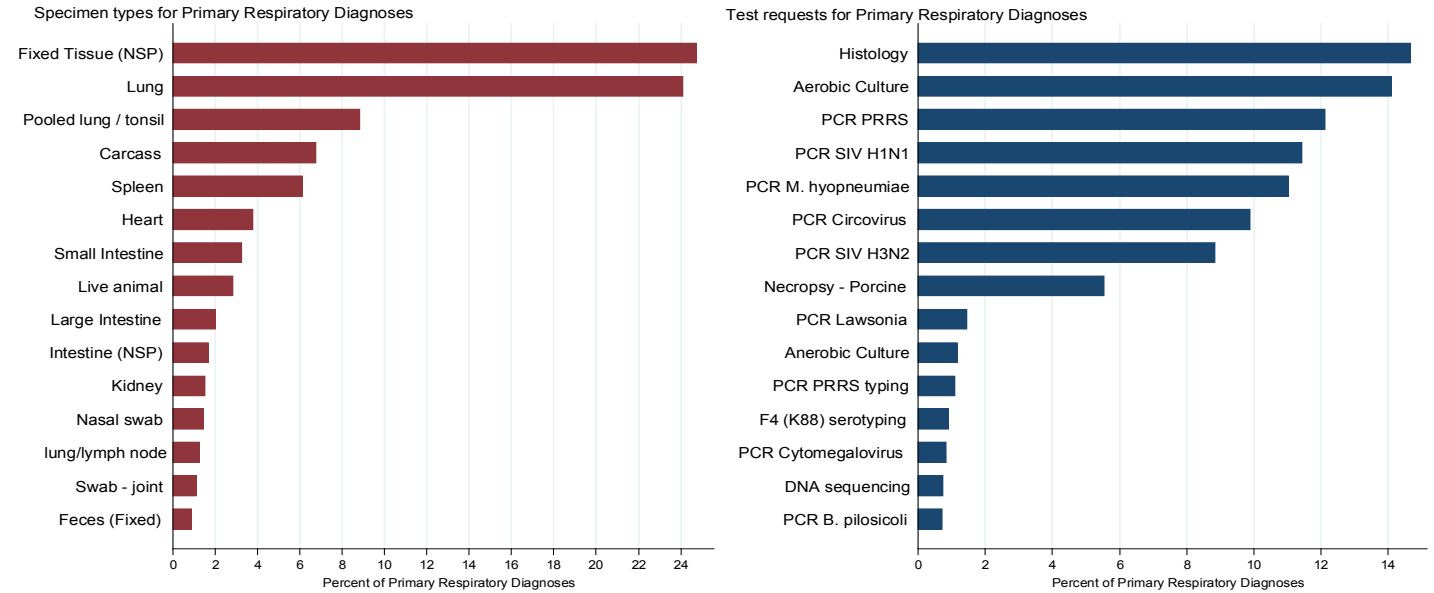
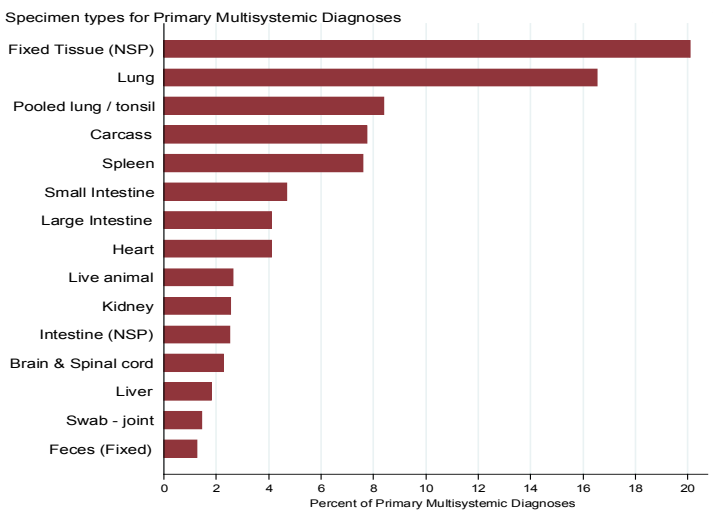
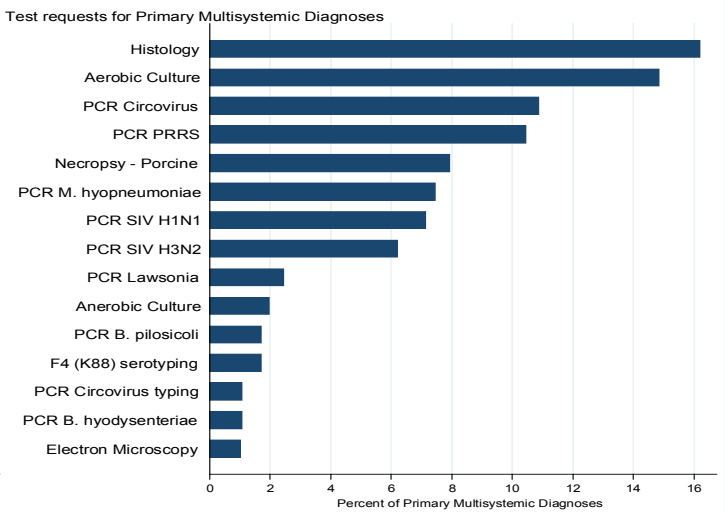
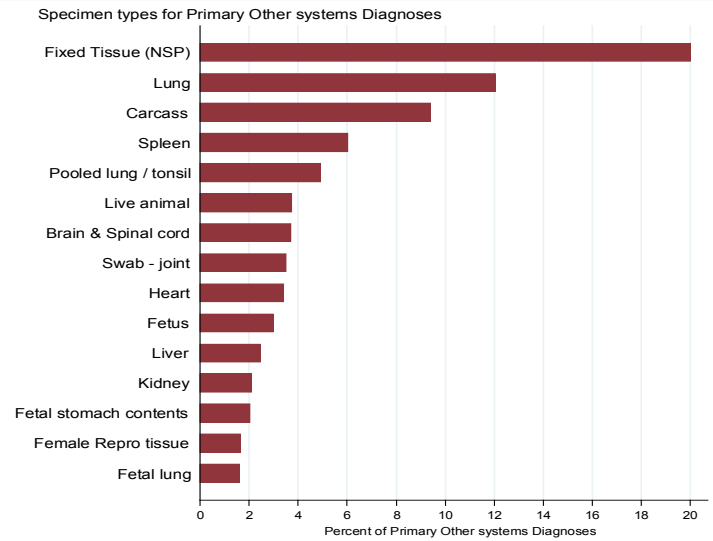
Appendix I: Specimen type and test request distributions by organ system group

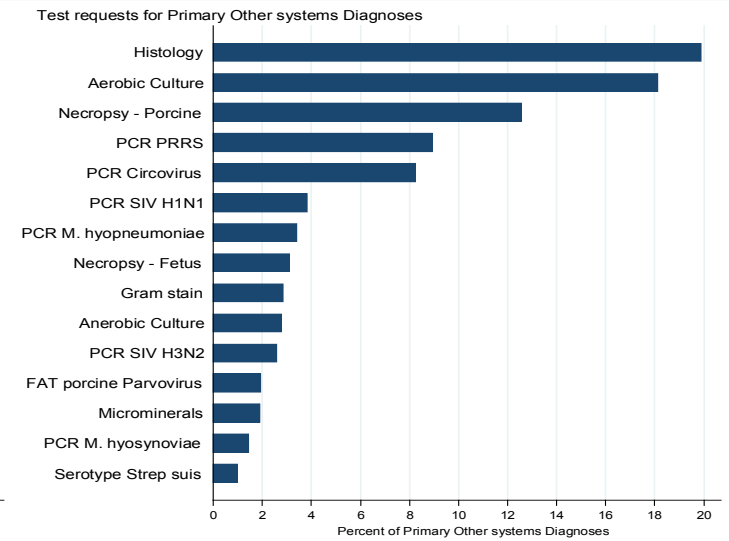





Specimen types for Primary Multisystemic Diagnoses
Fixed Tissue (NSP)
Lung
Pooled lung / tonsil
Carcass
Spleen
Small Intestine
Large Intestine
Heart
Live animal
Kidney
Intestine (NSP)
Brain & Spinal cord
Liver
Swab - joint
Feces (Fixed)
0 2 4 6 8 10 12 14 16 18 20
Percent of Primary Multisystemic Diagnoses


Test requests for Primary Multisystemic Diagnoses
Histology
Aerobic Culture
PCR Circovirus
PCR PRRS
Necropsy - Porcine
PCR M. hyopneumoniae
PCR SIV H1N1
PCR SIV H3N2
PCR Lawsonia
Anerobic Culture
PCR B. pilosicoli
F4 (K88) serotyping
PCR Circovirus typing
PCR B. hyodysenteriae
Electron Microscopy
0 2 4 6 8 10 12 14 16
Percent of Primary Multisystemic Diagnoses


Specimen types for Primary Other systems Diagnoses
Fixed Tissue (NSP)
Lung
Carcass
Spleen
Pooled lung / tonsil
Live animal
Brain & Spinal cord
Swab - joint
Heart
Fetus
Liver
Kidney
Fetal stomach contents
Female Repro tissue
Fetal lung
0 2 4 6 8 10 12 14 16 18 20
Percent of Primary Other systems Diagnoses


Test requests for Primary Other systems Diagnoses
Histology
Aerobic Culture
Necropsy - Porcine
PCR PRRS
PCR Circovirus
PCR SIV H1N1
PCR M. hyopneumoniae
Necropsy - Fetus
Gram stain
Anerobic Culture
PCR SIV H3N2
FAT porcine Parvovirus
Microminerals
PCR M. hyosynoviae
Serotype Strep suis
0 2 4 6 8 10 12 14 16 18 20
Percent of Primary Other systems Diagnoses